JOURNAL

OF THE

Elisha Mitchell Scientific Society

VOLUME 55
1939

Published for the Society
by the
University of North Carolina Press

CONTENTS OF VOLUME 55

HENRY VAN PETERS WILSON
1863-1939

JOURNAL

OF THE

Elisha Mitchell Scientific Society

Volume 55 June, 1939 No. 1

HENRY VAN PETERS WILSON

Memorial Resolution

Adopted by the Faculty of The University of North Carolina

In the passing of Dr. H. V. Wilson on January 4, 1939, the University has lost one of the ablest teachers it has had, the faculty one of the wisest and most thoughtful counselors, the students one of the most generally esteemed and respected instructors, and colleagues, students and members of the community generally a stimulating companion and a highly cherished friend.

Henry Van Peters Wilson was born in Baltimore, Maryland, February 16, 1863; educated in the public schools, including the Baltimore City College, where, to his enduring satisfaction, he had rigorous training in the classics, and then in the undergraduate division of The Johns Hopkins University.

After brief experiences in teaching, and in studies at a Medical School he reëntered The Johns Hopkins University to start upon the professional career from which there was thereafter no deviation. As a graduate student at Hopkins in the department made famous by Martin and Brooks, he was associated with a select group of exceptionally able young men, several of whom have since acquired high distinction in scientific research. Even before completing his graduate work, Wilson had acquired the particular respect and admiration of those around him for his notably thorough and precise research and for his wide reading and accurate analyses of literature and of observations.

Having obtained the degree of Doctor of Philosophy from Hopkins, Dr. Wilson held the Bruce fellowship of that institution for a year and

then served for two years as scientific assistant in the United States Fish Commission at the historic Fisheries Biological Laboratory at Woods Hole, Massachusetts. In this position he did not lose himself in administrative or routine duties or scatter his energies widely over practical fishery problems. On the contrary he undertook and carried to completion a masterly study of the embryology of the sea bass, the results of which were published in a monograph which has proved to be much more than a thorough meritorious piece of research; it has, indeed, frequently been used as a working model for graduate students.

In 1891 Dr. Wilson assumed his first, and one might say his last, regular teaching position when he was brought to Chapel Hill as head of a one-man Department of Biology. The only changes of title that came to him in the remaining forty-seven years of his career in the University were when the Department, after substantial growth under his guidance, was divided into Departments of Botany and Zoology, Dr. Wilson continuing as head of the Department of Zoology, when again in 1917 he was made Kenan Professor, and finally when, at his own request in 1935, he was relieved of administrative duties as head of the Department of Zoology. He continued as Kenan Professor, actively engaged in teaching, until the time of his death.

Almost his whole professional career was thus associated with this University and his success in teaching and in the administration of the Department and his achievements in research have played no insignificant part in establishing and spreading the reputation of the University of North Carolina for thoroughness and soundness of scholarship. When the young professor assumed the responsibilities of a Department of Biology in the University at Chapel Hill, the enrollment of students in the institution was less than 250, the revenues of the University were extremely restricted and the departmental budget was at a minimum. From the very beginning, Dr. Wilson determined that the Department of Zoology should not only be thorough in its work, but relatively broad in its offering and as well-equipped as the possibilities would permit. The number of courses he taught well and the amount of work he accomplished seem now remarkable. He offered courses in elementary physiology, in general biology, comparative anatomy or mammalian anatomy, embryology, medical histology, microtechnique and invertebrate morphology, besides occasional courses in general botany and systematic zoology. He accomplished all of this efficiently and continued to be productive in research only by intense concentration and the most systematic distribution of time and effort. He assumed also

a most meticulous habit with respect to the care and preservation of apparatus and material. In after years some friends have smiled at his regular habit of counting corks and vials and his measured dealing of cover glasses, pins and tacks, but it was through unfailing adherence to practices of economy that within a relatively short time the Department of Zoology came to have a body of equipment comparable to that of many institutions where the departmental budgets were two or three times as large.

Dr. Wilson's teaching, research and developmental activities were not restricted to Chapel Hill. Very early he began the practice of spending the greater part or all of his summers in the little seaport of Beaufort, North Carolina, already made famous in the biological world through the studies in marine biology that had been carried on there by Agassiz, Gill, Coues, Morse, Brooks and others. Through Dr. Wilson's influence the United States Fish Commission was soon led to establish a summer laboratory at Beaufort, and he became its first Director. The laboratory was conducted at first in a rented residence on the water-front and then for several summers in a leased warehouse adjoining the town wharf. One of Dr. Wilson's most esteemed colleagues, Professor J. A. Holmes, then State Geologist and lecturer in geology in the University, joined with him heartily and most effectively in the effort to put the United States Fisheries Biological Laboratory upon a permanent basis. So it was that in 1902 the first permanent marine biological laboratory south of New England came into being on a small island that had been presented to the Government by friends of Dr. Wilson, and with a building and plant largely as planned by him. The Laboratory, which continues in operation to this time, has been the temporary home of a great number of prominent zoologists, botanists and physiologists, who have found it a favorable place for the conduct of original research. It has also played a significant part in the exploration of fishery problems in the State and in the entire southeastern region. The fathers of this Laboratory were unquestionably Henry Van Peters Wilson, as the initiator and original director, and Joseph A. Holmes, whose practical vision and adeptness in public relations encompassed and solved the practical problems incident to its establishment.

It was in the Beaufort Laboratory that Dr. Wilson began most of his important research work, obtaining the material that was studied in spare hours and week-ends during the following winters in Chapel Hill. On a few occasions, while on leave, Dr. Wilson worked at other laboratories, notably Berlin and Naples, but by far the greater and the

more significant part of his research was done at Chapel Hill and Beaufort. Perhaps the most brilliant work accomplished by Dr. Wilson at these places was that dealing with the dissociation of cells and regeneration, a discovery and a technique which attracted world-wide attention and which lie at the very foundation of modern tissue-culture and experimental embryology. Even apart from this and the related researches which followed, Dr. Wilson would have won a permanent place in the annals of biological science for his studies of embryology and of the morphology, systematics and embryology of sponges.

Dr. Wilson was a member of the National Academy of Sciences; the Philosophical Society of America; the American Society of Zoologists, which he served as President in 1911; the Boston Society of Natural History; The American Association for the Advancement of Science (fellow); Société Linnéenne de Lyon; the Elisha Mitchell Scientific Society of which he was President in 1905-06 and again in the last year of his life; and the North Carolina Academy of Science of which he was President in 1912; he was representative of the American Society of Zoologists in the National Research Council 1929-32; and rendered editorial service at various times to the Journal of Morphology, Biological Abstracts and the Journal of the Elisha Mitchell Scientific Society.

It would not be appropriate here to catalogue his contributions to science nor would we attempt a complete analysis of his characteristics. We would, however, record our recognition and appreciation of some of the personal qualities which made him so effective as a teacher and so helpful to the institution. Dr. Wilson was notable for his extremely systematic habits, his great capacity for work, his clarity of thought and his lucidity and incisiveness in speech. He possessed a most highly developed critical faculty associated with his unyielding devotion to accuracy and to the best standards of life and thought and expression. He had a strong personal interest in good students and a lasting affection for them, which, it need scarcely be said, was reciprocated. Lacking patience with slovenliness of dress, speech, work, or thought, Dr. Wilson preserved inflexibly his high ideals for himself, for his students and for the University. Always courageous in expression of opinion, he never hesitated to condemn what he thought worthy of condemnation or to stand in the minority, or even alone, for that which seemed to correspond with his belief as to what was best. He was, indeed, no insignificant factor in making the University what it is today.

We have enjoyed his companionship, we have respected and admired his strength of character, his depth of conviction, his devotion to high

academic and personal standards. We mourn his passing, which leaves a distinct gap in our ranks. We extend our deepest sympathy to his children and to his sister and we felicitate them upon the memory of a father and brother who was master of his circumstances, distinguished in accomplishment and an exemplifier of the best in personal ideals.

WILLIAM DEB. MACNIDER,
FRANCIS F. BRADSHAW,
ROBERT E. COKER,
Committee for the Faculty.

SUPPLEMENTAL BIBLIOGRAPHY

There will be found in Volume 50 (1934) of this JOURNAL a list of Dr. Wilson's publications, 71 titles, while connected with the University from 1891 to 1933. In the following list are comprised: (1) His publications prior to 1891; (2) Certain omissions from the original bibliography; and (3) The titles of publications subsequent to 1933.

PUBLICATIONS PRIOR TO 1891

1886. Parasitic Cuninas of Beaufort. J. Hopk. Univ. Circ. **6**: 45.

1887. The Structure of *Cunoctantha octonaria* in the Adult and Larval Stages. Stud. Biol. Lab. J. Hopk. Univ. **4**: 95–107, 3 pls.

Trans. Metschnikoff on Germ Layers. Amer. Nat. **21**: 334–350, 419–433.

1888. Abstract of Observation on the Development of *Manicina areolata*. J. Hopk. Univ. Circ. **7**: 31–33, 2 cuts. Abst. J. R. Micr. Soc. 1888: 434–435; Amer. Nat. **22**: 355.

On the Development of *Manicina areolata*. J. Morph. **2**: 191–252, pls. 1–7. Abst. J. R. Micr. Soc. 1889: 231; J. Hopk. Univ. Circ. **8**: 39–40.

1889. On the Occasional Presence of a Mouth and Anus in Actinozoa. J. Hopk. Univ. Circ. **8**, no. 70: 37–38. Abst. J. R. Micr. Soc. 1889, pt. 6: 761.

Report as Bruce Fellow of the Johns Hopkins University. J. Hopk. Univ. Circ. **8**: 40.

On the Breeding Seasons of Marine Animals in the Bahamas. T. C.: 70.

1890. On a New Actinia, *Hoplophoria coralligens*. Stud. Biol. Lab. J. Hopk. Univ. Circ. **4**, no. 6: 379–387, pl. 43. Abst. J. R. Micr. Soc. 1890, pt. 3: 338.

OMISSIONS FROM BIBLIOGRAPHY OF 1934

1892. Non-Sexual Reproduction in Sponges. Amer. Nat. **26**: 626–628. Abst. J. Morph. **5**: 511–519.

1931. What Has Made the University of North Carolina What It Is? (Privately printed July; reprinted September)

RECENT PUBLICATIONS

1934. Cell Behavior in the Metamorphosis of the Halichondrine Sponge Larvae. Abst. J. Elisha Mitchell Sci. Soc. **50**: 50.

President Venable as a Colleague. Alumni Review **23** (3).

1935. Some Critical Points in the Metamorphosis of the Halichondrine Sponge Larva. J. Morph. **58**: 285–345, 4 pls.

1937. Notes on the Cultivation and Growth of Sponges from Reduction Bodies, Dissociated Cells, and Larvae. *In* Galtsoff: Culture Methods for Invertebrate Animals: 137–139.

1938. Behavior of the Epidermis in Sponges (Microciona) When Treated with Narcotics or Attacked by Aquarium Degeneration. J. Exp. Zool. **79**: 243–273, 2 pls.

THE MORPHOLOGICAL AND CYTOLOGICAL DEVELOPMENT OF THE SPOROPHYLLS AND SEED OF JUNIPERUS VIRGINIANA L.*

By Andrew Clark Mathews

Plates 1–9

I. HISTORICAL INTRODUCTION

Despite the fact that considerable investigation has been made on the morphological and cytological features of the more widespread species of the sub-family Cupressineae of the family Pinaceae, a complete and thoroughgoing story embracing both morphology and cytology does not exist. Since the life history of the eastern North American species, *Juniperus virginiana* L., is so little known, a treatment of this kind for this species is highly desirable. The inclusion of a morphological study of the small berry-like ovulate cone of this species is also particularly worthwhile in view of the fact that the homologization of the cone structure of the Cupressineae with that of the Abietineae has been a matter of considerable discussion among plant morphologists for a long period of time.

In 1907, Norén (47) gave a thorough historical account of the cytological work that had been done on *Juniperus* from the time of Hofmeister and Strasburger. Norén worked out the complete cytological story of *Juniperus communis*, including micro- and mega-sporogenesis, fertilization and the early elongation of the prosuspensor tier of the proembryo. Although Hofmeister (1851; 1858) made a few cytological studies of *J. communis* and *J. sibirica*, and Strasburger (1872, etc.) studied parts of the story of *J. virginiana*, no complete and detailed account with modern techniques had been made before Norén.

In 1909, Alice M. Ottley (48) published a cytological study of *Juniperus communis* and *J. virginiana*, and in 1910, Nichols (45) gave a cytological account of *J. communis* var. *depressa*. The major portion of Miss Ottley's study was on *J. communis*. Nichol's work was a

* A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Botany.

consideration of the American variety of *J. communis* and was very thorough. He went into more detail in regard to pollen tube development and especially the division of the body cell than Norén but to a lesser extent into megasporogenesis.

In 1933, Karl and Hally J. Sax (54) reported chromosome counts of representative conifers, including *J. virginiana*. The materials used for their study were chiefly the female gametophyte tissue and pollen mother cells in the two meiotic divisions of the latter.

No full account of the embryogeny of *Juniperus* has appeared since Strasburger, and his investigation which included *J. communis* and *J. virginiana* cannot be considered as entirely complete. Saxton and Buchholz have presented the greater part of our further knowledge of the embryogeny of the Cupressineae. Saxton (58) included a rather full account of this particular phase in the life history of *Actinostrobus pyramidalis* Miq. in 1913, while Buchholz presented the details of the embryogeny of *Biota* (*Thuja*) and *Libocedrus decurrens* in 1926 (7) and *Chamaecyparis obtusa* in 1932 (9).

Among the recent and outstanding contributions bearing on the morphological features dealt with in the present paper, Quisumbing's (1925) investigation of the seeds of conifers included a study of the stony layer development of *Juniperus virginiana*. Dluhosch (1937) studied *J. chinensis* in connection with his work on the development of the microsporophyll of the conifers.

The question as to whether the female cone of conifers is to be regarded as a simple or compound floral structure has been a subject of considerable discussion among morphologists for a long time. The studies and discussions that have been made of this question since 1827, in which year Robert Brown announced gymnospermy, are presented by Coulter and Chamberlain (1932, pp. 244–251). In 1926, Pilger (49) presented a thorough treatment of this question in his morphological account of the conifers in Engler-Prantl's *Pflanzenfamilien*, pp. 124–155. Since 1926, Lanfer (1933), Hagerup (1933; 1934), Hirmer (1936) and Propach-Gieseler (1936) have published rather exhaustive treatments of this subject. In all recent accounts, especially those of Hirmer and Propach-Gieseler, a comparative morphological study of the female cone-scale has been paramount. Miss Propach-Gieseler's paper presents a very detailed study of the earliest developmental stages of the cones of the Cupressineae, including several varieties of *Juniperus virginiana*, and her paper is, therefore, of great importance on this point.

According to Mohr (43), *J. virginiana* is native to eastern North America, extending along the Atlantic seaboard from Maine to Florida and westward to Minnesota and South Dakota on the north, and south to the vicinity of Austin, Texas, and the Gulf of Mexico. This species is exceptionally valuable for its timber, for wildlife food and protection, and for ornamental purposes.

II. MATERIALS AND METHODS

Juniperus virginiana is a dioecious conifer, and in order to obtain all the developmental stages in its life history, it was necessary to collect the female material from a site where male and female trees were growing close together. A clump of trees of both sexes was found near the campus of Duke University, Durham, N. C., and the majority of the female material for this study was collected there during the spring and summer of 1933. Slabs were cut from two sides of the flowers and developing cones with a razor blade and the material plunged immediately into the fixing fluid. Collections were made every two days beginning just prior to time of pollination, viz. about March 1st, until archegonial formation, about May 25th, and once a day and sometimes more often until after fertilization, viz. June 8th. The remainder of the female material was collected from trees in the Coker Arboretum of the University of North Carolina and from other places in Chapel Hill, N. C.

The male material was collected from trees on "Windy Hill" in Chapel Hill, N. C., and also prepared for fixation by cutting slabs from two sides of the cones. The collecting was begun in the fall of 1933, beginning about August 1st and repeated about every two or three days until September 30th. By this time the pollen grains were formed and only an occasional collection was made thereafter until about the time of pollination the following spring.

Several fixing fluids and modifications of them were used. The following formula, a modification of that suggested by Chamberlain (15, p. 343), served as a good general one for the female material:

A. 0.5 c.c. chromic acid
3.0 c.c. glacial acetic acid
100.0 c.c. distilled water
B. Add 1% osmic acid in proportion of one part to 15 parts of A at the time of fixation.

The hot alcoholic corrosive sublimate—acetic acid recommended by Miss Ethel Thomas (Chamberlain, 15, p. 343) gave unsatisfactory re-

sults with the late stages of the female gametophyte. The fixing fluids found most satisfactory for the male cones were the chrome-osmo-acetic acid solution recommended by Miss Ferguson (25) and the Chicago chromo-acetic-osmic solution of Chamberlain (15). The majority of the embryogeny material was fixed in formalin-alcohol as recommended by Buchholz (6). However, a part of the material used in the study of embryogeny was sectioned in paraffin.

Haidenhain's iron-alum haematoxylin with orange G as a counter-stain gave excellent results. In tracing the development of starch in the pollen mother-cell cytoplasm as well as the fate of the starch brought into the egg cytoplasm by the sperm cell, a special staining combination of the safranine (alcoholic) and gentian violet (aqueous) of Flemming's Triple plus a very strong iodine solution was found very helpful. The slides were taken through safranine and left about 10 minutes in gentian violet. Then, after two dips in water, all excess water was removed from around the sections, and the strong iodine solution dropped on and allowed to remain for 3–4 minutes. The iodine was then removed and the slide dehydrated as quickly as possible by dipping in 95% alcohol and absolute alcohol, dipped about twice in clove oil and then into xylol for clearing, and mounted in balsam.

In staining whole sections of mature berry-cones, excellent differentiation was obtained by staining overnight in alcoholic safranine, destaining with acidulated alcohol, and completing with Delafield's haematoxylin for about 20 minutes. As recommended by Buchholz (7), dissected proembryos and embryos were stained in Delafield's haematoxylin and mounted in diaphane.

III. THE STAMINATE CONE AND THE MICROSPOROPHYLL

In his recent paper on the development of the microsporophyll of the conifers, Dluhosch (22) has given a brief review of the history of our knowledge of the development of the microsporophyll of conifers. In 1845, H. von Mohl considered the male conifer cone as a single flower, bearing sporophylls each of which bears two or more sporangia. Parlatore (1868) regarded single sporophylls as flowers, and his view predominated until the work of Celakowsky (1897). Celakowsky's view was that the primary form of the conifer sporophyll is radiate as in *Taxus*, and that the pollen-bearing leaf has been transformed from the shield-radial form to the bilateral form, in which only the lower side of the leaf has remained fertile, or pollen-sac bearing. Goebel's (1923) view agreed essentially with that of Celakowsky. He held that

the microsporophylls of *Taxus* and the Cupressineae are shield-shaped, and in spite of varying conditions within single groups and even within single flowers, that this may be extended to all species of conifers.

Dluhosch (22) agrees with Goebel in regarding the primary structural form of the conifer microsporophyll to be shield-shaped and the origin of the sporangia as marginal. He also describes for the Cupressineae the subsequent development of a sterile outgrowth just above the sporangial primordia, which is more or less marked in the different genera. This outgrowth together with the distal sterile tissue of the original sporophyll ultimately gives rise to a sort of "false" or secondary shield or peltate form. Dluhosch also reports the occasional occurrence of two rows of sporangia on the fertile shield margin in *Chamaecyparis* and *Thuja*. In these genera, the median portion of the primitive primordium of the sporogenous tissue becomes sterile, and the two rows of microsporangia are differentiated out of the initials of the resulting sporogenous tissue.

At Chapel Hill, N. C., the staminate cones of *Juniperus virginiana* become recognizable early in August (Figs. 1, 2) before pollination and fertilization the following spring and summer. The microsporangia pass the winter filled with mature microspores (Figs. 11, 36). Figure 3 is an outline of a longitudinal section of a staminate cone of *J. virginiana* at the time when the microsporangia contain mature pollen mother-cells, and Fig. 4 shows a mature cone just before pollination. The mature cone is made up of four opposing rows of 3 to 4 shield-shaped sporophylls on the median cone-axis. Miss Ottley (48) reported that at Wellesley, Massachusetts, the microsporangia of *J. virginiana* pass the winter in the pollen mother-cell stage, and that the microsporangia of *J. communis* do not begin development until the spring of the season of pollination. Nichols (45) also found that the staminate cones of *J. communis* var. *depressa* at Hartford, Connecticut, are first recognizable in late summer and pass the winter in a more or less rudimentary condition.

In Chapel Hill, the development of the microsporangia of *J. virginiana* is accomplished in a little less than two months, viz. from early in August until about the latter part of September (Figs. 5–11; 13; 14). Fig. 5 shows a very young potential microsporophyll at the top of a young male bud (as Fig. 1) prior to the bud's being recognizable as a male cone. In this potential sporophyll the resin cavity has already appeared, and after examination of several of these very young cavities it was concluded that they originate schizogenously. This agrees with Haberlandt's (26, p. 516 and p. 522) conclusion concerning the resin

cavities of *J. communis*. A striking characteristic of the sporophyll as well as vegetative leaves of *J. virginiana* is the median resin canal in the abaxial side of the sporophyll just beneath the epidermis. This canal runs parallel to the long axis of the leaf, extending from near the tip of the leaf and ending blindly just above the resin cavity. The canal seems to be formed by a mere separation of the epidermal layer from the mesophyll layer. The mesophyll cells are very loosely arranged, containing a large quantity of intercellular air space between them. The majority of these cells are richly supplied with chloroplasts, but some are decidedly resinous in character, although some cells seem to contain both chloroplasts and resin. It was observed that these chloroplasts become filled with large starch grains before the end of summer as evidenced in part by Figs. 10 and 11.

In a very short time the sporangial primordia become recognizable on the abaxial side and basal end of the young microsporophyll (Fig. 6). This is shown by the outward projection of the sporophyll tissue at this point; and just beneath the epidermis the primary archesporial cells are recognizable by their larger size, larger nuclei, and denser cytoplasm. The archesporium represented by Fig. 6 consisted of three cells in a plate parallel to the epidermis. Meanwhile, the resin cavity has enlarged considerably, and the sporophyll has grown a little longer. The epidermal cells also begin to acquire some resin. This resin deposit persists during the remainder of the development.

Nichols (45) has reviewed the history of our knowledge regarding the origin of the microsporangium of many gymnosperms. He shows that Goebel (1881), Coulter and Chamberlain (18), and other investigators, notably Coker (16) and Coulter and Land (21), have shown that the development of the microsporangium in gymnosperms is essentially similar to that of the eusporangiate pteridophytes. *Juniperus virginiana* is in agreement with this latter view.

It is now easy to follow the further development of the microsporophyll (Figs. 7–9; 12). Norén (47) did not consider the origin and early development of the microsporophyll and microsporangia in the European *Juniperus communis*; Nichols (45) described in considerable detail the microsporangial development in *J. communis* var. *depressa* but not the early morphological development of the microsporophyll of this species. However, Dluhosch (22) included *J. chinensis* in his morphological treatment of the microsporophylls of the conifers. *Juniperus virginiana* agrees in all essential respects with Nichol's description of the sporangial development and that of Dluhosch on the development

of the microsporophyll of *J. chinensis*. In *J. virginiana* the microsporangium continues to grow and project outward, and very soon a second outgrowth of the sporophyll becomes recognizable distally with reference to the young sporangia and immediately adjacent to them (Fig. 7). This outgrowth early becomes somewhat flattened and tapered at its outermost point and continues its downward growth in close association with the microsporangia (Figs. 8, 9). It finally becomes the sterile shield-half of the mature peltate sporophyll (Fig. 12).

During this early evagination of the sporophyll-outgrowth, the plate of archesporial cells within the microsporangium has divided periclinally and anticlinally to produce more archesporial tissue centrally located and surrounded by a sub-epidermal layer of sterile sporogenous cells. By periclinal division of this sub-epidermal layer and concomitant divisions of the sporogenous cells the three sporangial wall layers shown in Fig. 8 result. The primary sporogenous cells which are ultimately formed become thus surrounded by a one-layered tapetum which is recognizable by its uniformity and dense cytoplasm, an intermediate layer of tabular cells, and the outer epidermal layer. The sporangial wall is thus considered to be comprised of two wall layers. This differs from the sporangial wall of *Pinus*, which has been shown by Miss Ferguson (25), Mathews (40) and others to consist of 3 to 5 layers of cells. Fig. 8 also shows that the sporophyll out-growth has elongated still further downward and around the sporangia. The construction of a sporangium just prior to meiosis of the pollen mother-cells is shown in Fig. 9.

IV. MEIOSIS OF THE POLLEN MOTHER-CELL AND FATE OF THE MICROSPORANGIAL WALL LAYERS

It is difficult to say how much time is consumed in the meiotic divisions of the pollen mother-cells to produce the tetrads of microspores, for different male cones on the same day may be in different stages of this process. The cones at the end of a given branchlet are not usually so far advanced in meiosis as those farther back from the end. Nichols (45) reports that this process is completed within a week in *J. communis* var. *depressa* and Norén (47) and Miss Ottley (48) did not report the time required. It would seem that the meiotic divisions of the pollen mother-cells in *J. virginiana* are accomplished in approximately a period of 2–4 days at Chapel Hill, N. C. The sequence of the meiotic activity is made easier to follow due to the fact that in the earliest stages the meiotic division is farther along in sporangia at the base of the cone

than at the cone apex; however, during the latter part of meiosis, several cases were noted in which this sequence seemed to have been reversed so that the more advanced stages of meiosis appeared toward the apical end of the cone.

Miss Ottley (48) did not go into the details of meiosis in *J. virginiana*, but Norén (47) and Nichols (45) made thorough studies of this in *J. communis*. The essentials of meiosis in *J. virginiana* are in close agreement with those described by these authors for *J. communis*; and as also borne out in part by these authors, the nuclear behavior at this time bears a striking similarity to that described by Allen (1) for *Lilium canadense*, Mottier (44) for *Podophyllum*, *Lilium*, etc., and Miss Ferguson (25) for *Pinus*. The mature pollen mother-cells are polyhedral, nearly isodiametric and approximately 20 microns in diameter (Figs. 9, 15, 38). The nuclei of the pollen mother-cells are about 10.5 microns in diameter and usually contain two conspicuous nucleoli. The measurements for *J. virginiana* are approximately 2 microns less than Norén (47) and Nichols (45) reported for *J. communis*; and the nuclei are only about one-third as large as those of *Larix* and *Pinus*. The chromatin material presents a finely reticulated appearance with small chromatin granules rather irregularly arranged along the linin threads. At some places two threads seem to lie beside one another as if beginning to show signs of pairing (Fig. 15), but this could not be positively demonstrated. Just prior to synizesis the chromatin threads have become quite conspicuously lumpy, and begin to be drawn in away from the nuclear membrane. Pairing of the chromatin threads is more readily discernible at this stage (Fig. 16 (1), 16 (2)). The threads continue to draw in more closely until they form a tight ball that usually lies at one side of the nuclear cavity. Figs. 17, 18, and 19 show progressive stages in the behavior of the nuclear material after synizesis. Nichols stated for the chromatin material at this point in *J. communis* that from observations made at the time of synizesis and from preceding and subsequent phenomena, "It may be inferred that there is present at first a series of double threads, and that toward the close of synapsis these coalesce and unite end to end to form a single bivalent spireme." This appears to be the case in *J. virginiana*. Gradually the spireme becomes transversely segmented; and the segments begin to shorten, thicken and become somewhat twisted (Figs. 20, 21). By this time, the nuclear membrane is no longer a definite thin membrane, but merely a wide band of dense fibrils which fade out into the surrounding cytoplasm. Fig. 22 represents a stage subsequent to the diakinesis stage of

Sharp (61). The chromatin segments are completely differentiated just prior to becoming arranged at the equator of the heterotypic spindle.

By this time the pollen mother-cells have lost their angular shape and are considerably more spherical and separated from one another. Nichols suggests that this is apparently brought about by the dissolution of the middle lamellae of the mother-cells in the manner described by Strasburger (1882).

Gradually the weft of fibers derived from the nuclear membrane together with fibers derived from the cytoplasm push their way into the nuclear cavity and constitute the spindle for the heterotypic division. Figs. 23 and 24 show the meta- and telophases of the heterotypic division. Upon arriving at the poles the chromosomes very soon become enclosed by a delicate nuclear membrane and begin to show considerable vacuolation and become somewhat irregularly distributed throughout the nuclear cavity. The mature resting daughter nuclei of the division are generally biconvex with the long axis perpendicular to the axis of the meiotic spindle (Figs. 25, 26). As pointed out by Coker (16) for *Taxodium*, Norén (47) for *J. communis*, and Mathews (40) for *Pinus palustris*, there appears to be a temporary cell plate laid down at the equator between the daughter nuclei of this division.

After a brief rest period the daughter nuclei of the heterotypic division undergo the second meiotic or homotypic division (Figs. 27, 28, 31). The two nuclear spindles may be variously oriented in the cell, but as noted by Nichols (45), tetrahedral arrangement seems to be more prevalent. The chromosomes are generally "V" or "U" shaped, short and thick, and somewhat irregular in outline. The cell plates laid down after this division are at first similar to that of the heterotypic division, but they gradually become thicker and are persistent (Figs. 28, 31). Gradually a deposit of what appears to be a mucilaginous layer begins to be laid down at the periphery of each of the four newly formed microspores of the tetrad. As noted by Norén (47) and Nichols (45), *Juniperus* does not possess as definite and rigid-appearing walls as Miss Ferguson (25) has described for *Pinus*. The fact that mucilaginous walls do exist is evidenced, however, by the microspores being definitely separated from one another by a narrow interval (Fig. 32); but these walls, if they may be considered as such, take orange G stain only to a slight extent, and they are of very short duration, since the general liberation of the microspores takes place very soon.

The other investigators of *Juniperus* have noted the difficulty of obtaining the chromosome count in meiosis. Norén (47) considered the

haploid number in *J. communis* to be eleven, while Nichols (45) was convinced after considerable study that the number in var. *depressa* was twelve. Karl and Hally J. Sax (54), working at the Arnold Arboretum, made chromosome counts for numerous conifers, including *J. virginiana*, *J. communis*, and *J. chinensis*. They used the aceto-carmine smear technique on mitoses of female gametophytic tissue and meiosis of pollen mother-cells. A resumé of their work shows the basic haploid number of chromosomes to be twelve for most gymnosperms, except the Gnetales. Exceptions to this fact, however, were found in the Cupressineae and Taxodineae in which the prevailing number was eleven, in *Pseudotsuga* thirteen and *Pseudolarix* twenty-two. According to these authors, "deviations from the primary basic number of 12 are attributed to the loss of a small chromosome, following translocation of segments, in the Cupressineae and Taxodineae; duplication of a chromosome in *Pseudotsuga*; and polyploidy in three species (including a variety of *Juniperus chinensis*) of Conifers." Some effort was made by the writer to obtain an accurate chromosome count in *J. virginiana* from polar views of the anaphase stage in the meiotic divisions of the pollen mother-cell. As accurate a count as was obtainable showed the haploid number to be 11 (Figs. 29, 30), which checks with the studies made by Sax and Sax.

As suggested by Mathews (40) for *Pinus palustris*, the liberation of the microspores from the tetrad seems to be effected also in *J. virginiana* by a dissolution (probably enzymatic) of both tetrad chamber walls and the old mother-cell wall which surround the tetrad (Fig. 33). The microspores have no developing air-sacs to effect a mechanical rupture as described by Miss Ferguson (25) for *Pinus*, and the general appearance of the liberation seems to be one of chemical dissolution. When the young microspores are liberated, they have the general shape of their tetrad chambers, are delimited by a delicate pliable membrane and possess one rather large resting nucleus which is at first devoid of a nucleolus (Figs. 33, 34). The microspore cytoplasm contains a rich supply of starch which in haematoxylin preparations gives it a marked frothy appearance. Very soon, however, the microspore begins to round itself off, and the thick exine and thinner intine walls begin to be laid down about the periphery of the plasma membrane (Fig. 35). The young liberated microspores are about 12μ in diameter, while their nuclei measure approximately 6.5μ. Before the beginning of the winter period of dormancy, the young microspore has grown to about 16.5μ, and the starch grains which it contains have enlarged considerably.

Meanwhile, the intine and exine walls become thicker. While the microspore becomes larger, its nucleus becomes smaller as it assumes its resting condition for the winter, its average size at this time being about 5 microns. Its chromatin threads have arranged themselves into a finely reticulated structure and the nucleus contains one definite nucleolus. There is no division of the microspore nucleus before pollination.

Particularly worthy of note is the development of starch in the cytoplasm of the pollen mother-cell. The investigators of *J. communis* have described the presence of starch in the developing tetrad of microspores, but their figures do not entirely agree with the condition in *J. virginiana*. It would seem unlikely that there should be much difference in these two species in this respect. Using a combination of Flemming Triple stain and strong iodine solution, the presence of starch was easily detected in the mother-cell cytoplasm at the different stages. (See figures of meiosis, Plate 2, especially Figs. 38–40.) The starch grains are small just before synizesis in the mother-cell, but they become rapidly larger, and at the close of the heterotypic division they are quite large and show a rather characteristic alternate arrangement above and below the temporary (ephemeral) cell plate (Figs. 25, 40). As noted by Nichols (45), throughout the entire development of the microspore tetrad the cytoplast shows a striking "alveolar" character. This is obviously due to its rich starch content. The starch grains of the young pollen grain are very large and occupy the major portion of the cell (Fig. 36). Each starch grain has two or more lamellae and a rather distinct hilum. This stage is found near the end of the growing season, namely, late in September. By the time of pollination the following spring, starch no longer is apparent in the cytoplasm of the pollen grain (Fig. 37). This is in accord with the investigation of J. Doyle and Clinch (24), who studied certain seasonal changes in leaves of *J. virginiana* and other conifers.

During meiosis of the pollen mother-cell and development of the young pollen grain, several changes have occurred concomitantly in the microsporangial wall. Figs. 9, 10, and 11 show the successive changes as they occur in the tapetum and inner and outer wall layers from the mature pollen mother-cell stage until the sporangium is filled with young pollen grains. The outer wall layer, which is epidermal in origin, and the tapetum contain numerous starch grains at the end of the heterotypic division of the pollen mother-cell and are the only prominent layers surrounding the mother-cells. The cells of the inner wall layer

have already become considerably stretched and crushed and are rapidly degenerating. By the end of September the majority of these cells have been completely absorbed and only an occasional remnant remains (Fig. 11). Meanwhile, only a few starch grains show up in the outer sporangial layer. At this stage, the tapetum contains very little starch, and its cells have begun to appear considerably degenerated. Just before pollination the following spring, the inner sporangial wall layer and tapetum have been completely adsorbed, and only the outer wall layer remains to constitute the microsporangial wall. The cells of this layer are rectangular with their long axes parallel to the long axis of the sporophyll stalk cells, and their internal walls show prominent cellulose thickenings (Figs. 12–14). These thickenings appear as strong bands which are seen in side view to alternate on either side of the cell extending from the top of the cell downward laterally to the mid-line at the bottom of the cell, thereby furnishing considerable strength to the sporangial wall and functioning in its dehiscence.

V. POLLINATION

Pollination in *Juniperus virginiana* occurs between the middle and last of February in the vicinity of Chapel Hill. The spring season was early at Chapel Hill in 1938, and pollination had been mostly completed on the trees of this species in the Coker Arboretum by February 10th, but had not begun on the trees at "Windy Hill" on this date.

The majority of the male cones bear sixteen sporophylls (Fig. 4), each sporophyll bearing 3–4 pendent sporangia on its lower surface. The microsporangial wall splits longitudinally on the lower side to discharge the pollen grains. This dehiscence is the mere separation of the rectangular sporangial wall cells in a line parallel to their longitudinal axes. Consequently, the line of dehiscence is parallel to the longitudinal axis of the sporophyll. When a ripe sporangium is pricked with a needle, there occurs a rather active sporangial dehiscence, and as the pollen grains are discharged, the edges of the sporangial wall curve backward from the point of rupture. It is very likely that the internal cellulose bands of the sporangial wall cells (Figs. 13, 14) play a large part in the natural dehiscence by being drawn when dry toward the mid-line of the cell (Fig. 14) and thereby creating considerable tension on the sporangial wall. These cellulose bands would seem, then, to serve in the same capacity as the thickened portions of the annular cells of the leptosporangiate fern sporangium.

As has been noted by other investigators, the micropyle of the ovule

is wide open at the time of pollination (Fig. 63), and, as probably first recorded by Jack (30) for *J. communis,* "from each orifice there is exuded a minute globule of clear, shining liquid which rests like an iridescent bubble on the tip and serves to catch the pollen and conduct it to the nucellus or ovule within." Lawson (37) also noted that this pollination-drop is observed more frequently during the early morning, before the heat of the day has caused evaporation. The pollen grains finally lodge in the saucer-like depression at the tip of the nucellus, and after a few days the micropyle is closed due to the elongation of the layer of integument cells lining the micropyle. These cells elongate in a plane transverse to the long axis of the ovule and are generally known as micropyle-closing cells (Fig. 82a). In paraffin sections made just after pollination, the pollination-drop shows at the nucellar tip as a flat, wrinkled mass, apparently of a mucilaginous nature (Fig. 82a).

VI. THE MALE GAMETOPHYTE

Early Development.—The history of our knowledge of the pollen tube of *Juniperus* begins with Hofmeister's (28) investigations of *J. communis.* He found that two years are required for the pollen tube development in this species, and at the time of fertilization he saw a large cell in division in the end of the tube and a great many small cells (about 10 are figured) above this one. Hofmeister (29) published another account of the pollen tube development and fertilization in *Juniperus* in 1858. This time he observed the division of the large cell of the pollen tube into two smaller cells (the sperm cells). According to his interpretation, these two cells produced by repeated divisions sixteen free cells which then passed over the archegonia, or four medium-sized or eight smaller spherical cells without very definite membranes, which probably arose from repeated divisions of a still larger initial spherical cell. However, Strasburger (65) was able to find only granular cytoplasm in the pollen tube of *J. communis.* In 1872, though, Strasburger (66) found in *J. virginiana* a large free cell which divided into two daughter cells before fertilization. In 1878, the same author (67) gave another and more accurate account of the pollen tube of *Juniperus,* but this time he seems to have misinterpreted the contents of two tubes as the contents of one. Belajeff (4) was the first to show the correct interpretation of this phase of development. He found that the young pollen tube divided into two dissimilar cells, and that the cell remaining behind in the pollen tube divided further into a cell and a free nucleus, the first preceding the second as they both moved

forward into the tube. In the tube, this cell was overtaken by the free nucleus with the result that a mobile group consisting of a cell and two nuclei was formed at the anterior end of the pollen tube. Finally, he observed that the cell of this mobile group later divided into two equal sperm cells, both of which might participate in fertilization.

During the winter rest period before pollination in *J. virginiana* the pollen grains have completely lost their starch content, their cytoplasm now being left filled with numerous large vacuoles, and their cell walls, especially the exine, having become much thicker (Fig. 37). The pollen grain nucleus is very definitely spherical, occupies a central position in the cell, and in no instance was observed to divide before pollination. The pollen grains are wingless, as is true of those of all other Cupressineae. Lawson (37) has shown that in the Cupressineae, with the exception of *Cupressus* and *Juniperus*, the first division of the microspore nucleus occurs before pollination, giving rise to the generative and tube nuclei. He also shows that the Cupressineae are characterized by the absence of vestigial prothallial cells or of nuclei representing such cells.

In *J. virginiana*, pollination and fertilization take place during the same growing season, a little less than four months apart. This species agrees with most gymnosperms in this respect, but differs from *J. communis* (Norén, 47; Ottley, 48; Nichols, 45) and *Pinus* (Ferguson, 25; Mathews, 40) in that fertilization follows the next season after pollination in these species.

According to Nichols (45), the division of the primary pollen grain nucleus in *J. communis* to form the generative nucleus and pollen tube nucleus occurs within a week after the pollen grain reaches the tip of the nucellus, the generative nucleus becoming surrounded immediately by dense cytoplasm and separated from the "pollen cell" by a thin plasma membrane. In *J. virginiana*, very soon after this division, the exine wall ruptures and the intine wall is pushed outward to accomplish germination. Fig. 41 shows a pollen grain at the tip of the nucellus; and stages of germination nearly a month later are shown in Figs. 42 and 43. In these stages the lenticular generative cell is still in the extreme pollen-grain-end of the young pollen tube, while the pollen tube nucleus has begun to migrate downward in the tube. In Fig. 42, the pollination-drop is shown collapsed at the tip of the nucellus. In the section shówing the pollen tube of Fig. 43, the ruptured exine wall was located just above the pollen grain end of the tube. Near the base of the nucellus the megaspore mother-cell is at this time in its resting stage or in a very early stage of its meiosis (Figs. 82a, 82b).

The pollen tube now begins to push its tip between the cells at the nucellar tip; meanwhile the generative cell enlarges and rounds up, and the tube nucleus moves nearer the end of the tube (Fig. 44). Some of the nucellar cells are crushed and the protoplasts of nearly all the cells immediately adjacent to the tube become permanently affected, if not destroyed. All the young pollen tubes observed showed the pollen-grain-end of the tube somewhat protruded above the nucellar tip (Fig. 44). The elongation of the young tube while it is within the pollination-drop and before it reaches the nucellus may be the explanation of this condition. In *J. communis*, the developing male gametophyte passes the winter with tube contents nearly similar to those shown for *J. virginiana* in Fig. 44.

While the spherical generative cell is still in the pollen-grain-end of the tube, its nucleus divides to give rise to the body cell nucleus and stalk cell nucleus. In *J. communis*, Nichols (45) shows that these two nuclei, of which the body cell nucleus is the smaller, at first lie free in the cytoplasm of the tube, while the body cell nucleus soon becomes invested with a zone of dense cytoplasm which develops a membrane, and that a true stalk cell is never formed. While this division was not observed in *J. virginiana*, a body cell with a dense cytoplast and only a stalk cell nucleus result from the division of the generative cell. These promptly move down the pollen tube and come to lie near the tube nucleus a short distance from the tip of the tube (Fig. 45). Each of the three nuclei of the pollen tube has a single well-defined nucleolus and the chromatin material is evident in each nucleus as a very delicate reticulum. The stalk nucleus becomes closely associated with the pollen tube nucleus very soon after being formed, and it is and usually remains smaller than the latter. The pollen tube elongates only to about one-third the length of the entire nucellar cap when it ceases further elongation for approximately a month. Fig. 46 shows the contents of a pollen tube which had penetrated about one-third of the nucellar cap and was about a month older than that shown in Fig. 45. The body cell nucleus has grown nearly twice its former size, and the cell has acquired a considerably larger cytoplast. Thus it is in the 3-celled stage in *J. virginiana* and not the 2-celled stage as in *J. communis* that the pause in the elongation of the pollen tube occurs. Accompanying the increase in size of the body cell, its cytoplasm gradually becomes denser and the cytoplasmic granules become larger. Later developmental stages reveal the numerous granules contained in the body cell cytoplast at this time to be starch. Since the pollen tube cytoplasm is entirely devoid of starch and the nucellar cells are abundantly supplied

with it, it is very likely that the subsequent enlargement of these granules before fertilization is due to the translocation of starch from the nucellar cells to the body cell cytoplasm.

During this pause in the elongation of the pollen tube, the megaspore mother-cell at the base of the nucellus undergoes meiosis and the functional megaspore gives rise to the massive free-nucleate megagametophyte, which in turn becomes cellular and the cellular megagametophyte ultimately bears a group of very young archegonia, before any further marked elongation of the pollen tube occurs.

During the latter part of May, the pollen tube again becomes active and forces its way through the nucellar tissue, crushing and disorganizing the cells of the nucellus with which it comes in contact and within a few days enters the archegonial chamber where its tip presses down close to the necks of the archegonia (Fig. 47). By this time, the body cell, which usually becomes somewhat elongated during its downward migration, begins to round up and finally to become quite spherical after it comes to rest just above the stalk and pollen tube nuclei in the end of the tube. Its cytoplast is now very dense and surrounded by a thick plasma membrane.

Division of the Body Cell.—Miss Ferguson (25) described the cytology of the body cell mitosis in *Pinus*, showing that there are formed two unequal sperm nuclei both occupying a common cytoplast in which also both stalk cell and pollen tube nucleus may or may not be included. The larger sperm nucleus is, as a rule, in advance of the other in the pollen tube. Coker (16), working on *Taxodium*, was the first to give an accurate description of the cytology of the division of the body cell for a species which behaves similarly to *Juniperus*. Nichols (45) treated this point in considerable detail for *J. communis*. This author stated that the body cell of *J. communis* divides about four days after the pollen tube enters the archegonial chamber and about three days before fertilization. Nichols also showed for *J. communis*, as did Coker (16) for *Taxodium*, that this division in the species studied usually occurs simultaneously with that of the central cell of the archegonium. Two equal cells are usually formed in these species, and like the body cell, each is bounded by a definite membrane (Fig. 48). Nichols showed for *J. communis* that these cells are at first hemispherical and lie close together, but after separating become approximately spherical. In *J. virginiana* they usually lie side by side as if the spindle of the body cell mitosis had been perpendicular to the long axis of the pollen tube (Fig. 48). The average diameters of the body cell and sperm cell are

approximately 57 microns and 44 microns, respectively. A mature sperm and a fragment of its mate are shown in Fig. 49. The nucleus has a thick membrane, a coarse reticulum of linin threads which bear irregular deposits of chromatin, and usually one rather large and well defined nucleolus. Like the body cell, the sperm cytoplast contains a dense aggregation of minute starch grains, but the cytoplasm does not show a radiate structure as described by Coker (16) for *Taxodium* and Norén (47) for *J. communis*. This feature of *J. virginiana* is also indicated for *J. communis* by Nichols.

Contrary to the type of spindle described by Miss Ferguson (25) for the body cell of *Pinus*, which is extranuclear and unipolar, Nichols (45) shows that in *J. communis* spindle fibers "originate entirely within the nucleus." Other details pointed out by Nichols are: 1. The body cell usually loses its spherical shape as it approaches division and becomes ovoid. 2. There is present within the nucleus a delicate protoplasmic network in addition to the chromatin and linin proper. 3. The coarse, anastomosing reticulum, in which chromatin cannot well be distinguished from linin, resolves itself into a slender, uniformly distributed spireme which shows beautifully a distinction between linin and chromatin; and the chromatin granules are very clearly arranged in pairs at fairly regular intervals along the lighter staining linin band. 4. The spireme segments into the haploid number of slender and often twisted chromosomes; simultaneously delicate granular fibers arise within the nucleus. 6. The chromosomes rapidly become shorter and thicker, soon orienting themselves at the equatorial plane where the fibers give rise to a blunt, multipolar diarch spindle; the nuclear membrane disappears.

The production of more than two male cells (or the functional equivalent of two male cells) by a single pollen tube has been described for several species of gymnosperms, including some of the Cupressineae. Some phylogenetic speculations have also been based on this point. To give a complete account of this matter from our present knowledge of the Cupressineae, we find that the majority of this sub-family seems to show striking agreement with *Taxodium* (Coker, 16) and *Cryptomeria* (Lawson, 36) of the Taxodineae in bearing normally only two equal male cells in the pollen tube. A striking similarity also exists in the general shape and character of the male cells in these plant groups. These facts have been borne out in the following species and genera: *Thuja* (Land, 32; Lawson, 37), *Libocedrus decurrens*, *Cupressus* (two species), and *Chamaecyparis* (Lawson, 37), *Juniperus communis* (Norén,

47; Ottley, 48; Nichols, 45), *J. virginiana* (Ottley, 48), *Widdringtonia cupressoides* (Saxton, 56), *Callitris* (Saxton, 57), *Actinostrobus pyramidalis* (Saxton, 58), and *Tetraclinis articulata* (Saxton, 59). The writer has found no indication of the presence of more than the two male cells in the tube of *Juniperus virginiana*.

In spite of the fact that two male cells in a single pollen tube seems to be the rule in the Cupressineae, Norén, Nichols, and Saxton have reported exceptional cases in which more than the usual two sexual cells and two sterile nuclei were present in the tube. Norén (47) described the occurrence of a large body cell with three nuclei in the pollen tube of *Juniperus communis*; Nichols (45) found several cases in which more than two male cells had been formed by the division of the body cell in the tube of *J. communis* var. *depressa*; and Saxton (57) reported a case in *Callitris verrucosa* in which "a large nucleus of quite a different character" was present in the pollen tube in addition to the usual contents. While these cases can only be considered as very exceptional, Juel (31) found several male cells (as many as twenty) in a single pollen tube to be the rule in *Cupressus goveniana*, and Doak (23) reported a complex of several male cells in most cases in *C. arizonica*. (Doak also states that the male cells of *C. arizonica* have a distinctive pear-like shape suggestive of motility.) On the other hand, Lawson (37) found that in two other species of *Cupressus* examined by him more than two male cells are never formed in a single pollen tube. Apparently the only other cases among the investigated gymnosperms in which the production of more than two male cells by the body cell has been reported are *Microcycas* (Caldwell, 11), in which the body cell regularly produces sixteen or more sperms; and *Ceratozamia* (Chamberlain, 14), in which four sperms are formed occasionally.

VII. MORPHOLOGICAL DEVELOPMENT OF THE OVULATE STROBILUS

The female flowers of *Juniperus virginiana* are first recognizable about the middle of September before pollination and fertilization take place the following spring. They are borne terminally on short axillary shoots from small branches produced during the current season (Figs. 50a, 50b). The female sporophylls are not recognizable as such until a few weeks subsequent to pollination, namely about the last of March; but at least two pairs of the female sporophylls grow up over and gradually coalesce above the one or two ovules to form the dark blue berry-like fruit. (The blue color of the berry-cone is normally obscured by a gray "bloom" which covers its surface.)

Pilger (49), in *Die Natürlichen Pflanzenfamilien*, simply states regarding the structure of the berry-cone that (1) the cones of the Sabina Section of the Juniperoideae are as a rule formed from six scales and (2) that the berry-cones of *J. virginiana* contain 1-2 seeds. In view of the fact that Miss Charlotte Propach-Gieseler (51) has very recently published an account of the developmental details of the female flowers of the Cupressineae, including four varieties of *J. virginiana*, it was deemed desirable to compare the species treated in the present paper with this author's results.

The majority of the female flowers of *J. virginiana* are composed of a very short axis bearing two pairs of opposing sporophylls which are designated from the cone apex downward as pair *a* and pair *b*. Exceptions to this are: (1) The growing-point of the shoot may extend high enough above the usual level to produce an additional pair of sporophylls, pair *a'*; and (2) the pair of scale-like leaves below pair *b* often becomes fleshy in berry-cones bearing two ovules and fuses with the sporophylls above to construct the ultimate berry-cone. Invariably, however, sporophyll pair *b* alone shows fertility, usually only one member being fertile but sometimes both members. A more extensive study into the gross morphology of the female flowers of this species shows, furthermore, that there are three well-defined flower-types. These are treated here under types A, B, and C. Flower-type A: This flower-type contains only one ovule which is borne in the axil of one member of the sporophyll pair *b*, designated sporophyll $\underline{b}$. Approximately two-thirds of the female flowers are of this type. Figs. 51 and 52 show the general structure of this flower-type, while Figs. 55 and 56 are diagrammatic transverse sections of the flowers shown in Figs. 51 and 52, respectively. Flower-type B: Approximately one-half of the remaining one-third of female flowers bear two ovules side by side in the axil of only one member of sporophyll pair *b*, while the other sporophyll of the pair is sterile. This type is represented in Figs. 53a and 53b; Figs. 57 and 58 are diagrammatic transverse sections of this flower-type. Flower-type C: The remaining one-sixth of the female flowers are of this type and are characterized by each member of sporophyll pair *b* being fertile and bearing a single ovule in its axil. Figs. 54 and 59 show the gross structure of this flower-type. The fertile sporophyll (or sporophylls) is never constant as to its circumferential position on the cone-bearing branch in all three of these flower-types. Furthermore, while no flowers of *J. virginiana* were found to bear more than two ovules, Miss Propach-Gieseler (51) describes the closely re-

lated species, *J. sabina*, as having 1–4 ovules with one member of the sporophyll pair *b* bearing only one ovule in the 3-ovuled flower and each member of this pair bearing two ovules in the 4-ovuled flower. In *J. keteleri*, whose flowers bear 2–6 ovules, the sporophyll pair *c* may be fertile.

In the maturation of the berry-cone, an outgrowth develops on the morphological upper side of the cone-scale and is termed "fruit-scale" by Hirmer (27) and Miss Propach-Gieseler (51), while that portion below the fruit-scale bearing the "point" is termed "cover-scale" by these authors. It is due largely to the profuse up-growth of the fruit-scales and their final coalescence above that the ovule (or ovules) becomes entirely enveloped in the mature berry-cone. The sporophyll-outgrowth of the fertile sporophyll contributes considerably more to the fleshy tissue of the berry-cone than its sterile neighbors. Figs. 60a, 60b and 60c represent different views of a mature berry-cone developed from Flower-type A described above. The dotted line indicates the outline of the seed. In this type the cover-scale tip of fertile sporophyll *b* is solitary on one side of the fruit, and the seed is borne in its axil. The cover-scale tips of the three sterile sporophylls are in a group directly on the opposite side of the berry-cone. A mature berry-cone produced from the Flower-type B is represented in Fig. 61. Here the arrangement of the cone-scales is principally the same as in Flower-type A, except that the fertile sporophyll *b* bears two seeds in its axil and the sterile sporophyll pair *c* usually enters into the construction of the berry-cone. The berry-cone resulting from the Flower-type C is different in shape from the other two types, being somewhat flattened on the sides of sporophyll pairs *a* and *c* and in general obovoid, whereas the other types are usually simply ovoid (Fig. 62). In the fruit of flower-type C, the sporophyll members of the pairs *a*, *b*, and *c*, which usually coalesce to form the fleshy cone of the fruit, directly oppose each other morphologically as do the vegetative leaves immediately below. Each member of sporophyll pair *b* bears one seed in its axil, and sporophyll pair *c*, as in Type B, takes a very minor part in the constitution of the fleshy fruit coat.

As the epidermal cells of the opposing sporophyll-outgrowths (fruit-scales) come in contact with each other above the ovule, they become somewhat elongated in the direction of the opposing fruit-scale. The final fusion is accomplished by an alternate interlocking of these epidermal cells of opposing sporophylls in a manner somewhat similar to the enmeshing of two cogwheels (Fig. 68). All the epidermal cells of

the sporophyll tissue are cutinized, but those of the epidermis outside the berry-cone have a thicker cuticle than those at the point of fusion and those lining the inside of the sporophyll coat.

VIII. NOTES ON THE COMPARATIVE MORPHOLOGY OF THE OVULATE STROBILUS

The ovulate cone of *Juniperus* differs from that of other Cupressineae in the coalescence of its fruit-scales around and above the ovule, or ovules, to form a solid and simple berry-like cone. Miss Propach-Gieseler (51) has shown that the development of all the Cupressinean female cones is essentially similar. Fertile scale-pairs usually occupy a rather central position on the cone-axis, above and below which lie sterile scale-pairs, no sterile pair occurring between fertile scale-pairs. It may be noted from Figs. 51–62 above that in *J. virginiana* sporophyll whorl (pair) *b* is fertile as a rule, while whorls *a* and *c* are sterile. The ovule grows out of a meristematic tissue which lies in the lower part of the morphological upper side of the fertile sporophyll. Usually the ovule is formed at the base of the megasporophyll which bears it.

As described above, the fertile cone-scales of *J. virginiana* as well as the normal sterile ones are 2-parted, consisting of the cover-scale and fruit-scale (Figs. 63–66). According to Hirmer and Propach-Gieseler, this is true of all the conifers. Hirmer regards the cover- and fruit-scales of conifers as arising by a serial splitting of the primitive megasporophyll primordium. While the cover- and fruit-scales of *J. virginiana* appear as a single unified organ (the cone-scale), the fruit-scale at the time of pollination appears as a slight bulge on the adaxial side of the cone-scale (Fig. 63). Even at this early stage the fruit-scale and cover-scale are each supplied with a separate vascular bundle, and this condition persists throughout the development of the cone (Figs. 63–66; 79).

Miss Propach-Gieseler (51) has shown that the Cupressinean cone closes its apical development with a sterile leaf structure, for which she suggests the use of the term "Akrokon." This term is taken from the shape of the fertile shoot which is narrowly conical and which together with the upper scale-pair forms the akrokon. In the older stages, however, the growing-point becomes broad and flattened, and the last-formed scales have extended over it and so closely drawn together at their bases that the growing-point is no longer distinguishable; the uppermost pair of scales alone represents the akrokon.

The same author has shown furthermore that the mature ovulate

cones of *Juniperus* do not differ essentially from those of other Cupressineae. The number of ovules of the section Sabina varies from 1-6. In *J. virginiana* there is usually only one ovule which is located at the base of (and adaxial to) a scale of whorl *b*; but because of an upward extension which takes place in the scale-pair *a* at the top of the shoot, this pair is pushed to the summit of the cone. The three ovules of *J. communis* are not borne in the upper megasporophyll whorl in whose axils they stand, but opposite the joints of the lower scale whorl (*b*), which agrees with the condition in the other genera. Here as elsewhere, except in *Cupressus goveniana*, the uppermost scale-whorl (*a*) represents the akrokon.

It is beyond the scope of the present paper to enter into a discussion of the interpretation of the floral-type (viz. simple flower vs. compound inflorescence, etc.) of the ovulate cone of *J. virginiana*. Gymnosperm morphologists seem fairly generally agreed that the male conifer cone represents a simple flower structure; but the female cone with its 2-parted cone-scales has been more of a problem to them. Pilger (49) has compiled a comprehensive account of the older theories bearing on this point. But among the recent investigators and theorizers are Lanfer (33), Hagerup (1933, 1934; see 34), Hirmer (27), and Propach-Gieseler (51). The last named conducted her detailed morphological study of the Cupressineae under the direction of Hirmer. Suffice it to say here that with very exhaustive investigations on which to base their interpretations, Lanfer and Hagerup adhere to the view that the female conifer cone is a compound inflorescence, while Hirmer regards it as a simple female flower. The papers of Hirmer and Propach-Gieseler are remarkable for their profuse illustrations, including photomicrographs and diagrams of the early development of cones of representative groups and a considerable amount of comparative morphological data. Hirmer traces the origin of the typical conifer cone and sporophyll unit back to the fossil lycopod-like Sphenophylls. In the opinion of this author, the single elements of this flower have split serially, thereby cutting off an adaxial (fruit-scale) and an abaxial (cover-scale) segment. Both of these segments are supplied by a separate vascular strand, but only the adaxial segment is ever fertile and bears a megasporangium or megasporangia. This fundamental plan, according to Hirmer, exists throughout the conifers, and he points out that it is not difficult to understand how by lateral fusions of the vegetative (abaxial) segments of all the sporophyll units of a cone, accompanied by certain concomitant changes

of the fertile (adaxial) segments internally the angiospermous ovary may have had its phylogenetic origin.

IX. THE STONY LAYER

Coulter and Chamberlain (20) say concerning the developing seeds of the Pinaceae that "the integument differentiates into . . . three layers . . . ; but the outer fleshy layer does not deserve its name, for it is represented only by a thin layer of cells that disappears with the maturing of the testa. The middle or stony layer in this case is the conspicuous one, the seed being said to ripen dry; while the inner fleshy layer is most largely and distinctly developed, as usual, in the free portion of the integument." Conforming to this description, the integument of *J. virginiana* differentiates itself into three layers. The middle stony layer has a very striking mode of development and is a very prominent part of the mature seed. It is also primarily due to the development of the stony layer that the integument finally becomes differentiated clearly into its three layers. Perhaps the most outstanding paper treating this subject is Quisumbing's (52) comprehensive study of the stony layer of seeds of gymnosperms, which includes the seed of *Juniperus*. The following account is deemed a worthwhile addition to Quisumbing's description.

About May 1st, when the ovule contains a free-nucleate megagametophyte at about the 64-nucleate stage, the secondary thickening which characterizes the stony layer first becomes recognizable in the micropyle-closing cells above the nucellar tip (Fig. 70). Quisumbing (52) states that this secondary thickening first shows up as late as the early appearance of the archegonial initials. The cell marked "X" in Fig. 70 is about the first of the middle layer of cells below the micropyle-closing layer to show noticeable secondary thickening. As was noted by Quisumbing, the order of progress of this secondary thickening of the integument cells continues downward through the middle region of the integument from the micropyle-closing layer. This can be traced in the diagrams and camera lucida figures 66, 67, 69, 70, 71a, and 79. Gradually, as the cells lower down the integument begin to show secondary thickening, the number of the integument cells which is to give rise to the inner papery layer increases. Sections of early stages in stone cell formation show the secondary deposit to be laid down on the inner surface of the thin primary wall to form a distinctly reticulated pattern. The phloroglucin and hydrochloric acid test shows this deposit to be

lignin. As the lignification of this middle integument tissue continues downward around the ovule, the deposit higher up in the young stony layer gradually becomes thicker. The larger spaces in the reticulation become smaller and smaller as more lignin is deposited (Figs. 71a, 71b, 72–78), so that finally only small orifices remain open to the interior of the well-defined pits in the thick stone-cell wall (Figs. 75–78). The cytoplasts of these stone-cells apparently remain quite normal and active throughout the entire lignification period, and indeed, their nuclei are still conspicuous in mature stone-cells of berry-cones collected August 5 (Figs. 77, 78, and 80). It is not unusual to find tiny strands of cytoplasm projecting from the periphery of the cytoplast toward and into the pit orifices (Fig. 76). The pits are long and narrow and apparently at times are branched (Figs. 77, 78).

Finally, about June 6th, the middle portion of the integument has become completely differentiated by the lignification process (Fig. 69). A longitudinal section of the nearly mature fruit collected on August 5th shows the thick stony layer to be a very rigid one of stone cells nearly uniform in thickness except at the mid-region of the seed where it is somewhat thicker and at the loci of the resin cavities where it is a little thinner than elsewhere. This lignification of the middle tissue of the integument brings about a striking change in the orientation of the axes of the cells comprising the integument, as pointed out by Quisumbing (52). Before lignification sets in, practically all the integumentary cells except the epidermis and the outer "fleshy" layer, which are elongated parallel to the surface of the developing seed, are nearly isodiametric and quite similar in general appearance. But very soon after the beginning of the lignification process the lignified cells become elongated perpendicular to the surface of the seed, and this manner of orientation persists to maturity. During this development, the cells of the inner papery layer become more numerous and those lying adjacent to the stony layer become somewhat pushed inward and crushed by the stony layer, thus forming a distinct line of demarcation, when viewed under high magnification (Figs. 71a, 72, 73, 80). A radial section about half-way between the top of the mature seed and its equator shows the integument to consist of the inner papery layer eight cells in thickness, the stony layer of about eight cell-layers and the outer "fleshy" layer of only one cell-layer plus the epidermal layer (Fig. 80). A detailed study of the stony tissue of the mature seed coat shows the inner- and outermost layers of these cells to be largely isodiametric and the intervening transversely elongated cells somewhat

tapered at their ends. Fig. 79 is a diagram of a longitudinal section of these three integumentary layers in their final proportions.

X. THE MEGASPORANGIUM

The Megaspore Mother-cell.—The development of the megaspore mother-cell, the megasporangium and the megaspores in *J. virginiana* is in accord with the descriptions by Norén (47) and Nichols (45) for *J. communis*. The ovulate flower-buds are formed in the axils of the leaves of only the young branches of the current season. They first appear in the latter part of the growing season prior to pollination. Although these buds are not recognizable to the naked eye until about a week before pollination, viz. about Feb. 15, dissected lateral buds on branches produced the season prior to pollination show young ovules already developed (Fig. 50b). At first, the integument extends high above the small nucellus; but the nucellar cells divide rapidly to form long parallel rows, and finally considerable differentiation takes place at the base of these long rows of nucellar cells to form a spherical mass of centrally placed cells at the base of the nucellus. There are at least 10-12 of these cells, and they constitute the female archesporium (Fig. 81). These archesporial cells and their nuclei are a little larger than the other nucellar cells, and they possess a denser cytoplast and 1–2 well-defined nuceoli (Fig. 81). The nucellar (vegetative) cells surrounding the archesporium become somewhat flattened and form more or less concentric layers. In *J. virginiana*, the archesporium is formed early in March before the formation of the cellular megagametophyte about May 25. This is at variance with the condition in *J. communis* which produces its archesporium about 12 months prior to megagametophyte formation and passes its winter after pollination in the archesporial stage (Nichols, 45). The ovules of *Pinus Strobus*, etc. (Ferguson, 25) and *P. palustris* (Mathews, 40) pass their first winter after pollination in an early free-nucleate (usually 32) megagametophyte stage.

Development of the Megaspores.—As Nichols (45) pointed out, it is impossible to predict with certainty which cells of the archesporial tissue will become the megaspore mother-cell, but it is usually one of the most centrally placed cells. In *J. virginiana*, the megaspore mother-cell becomes differentiated about March 20 (Fig. 82b) and just after germination of the pollen grains at the nucellar tip (Fig. 82a). This cell is considerably larger than the other archesporial cells. Its cytoplasm is rather dense and contains the conspicuous kinoplasmic body just below

the nucleus as described by Norén (47) and Nichols (45) for *J. communis* and for *Taxodium*, *Thuja*, and *Taxus* by Coker (16, 17), and *Torreya* by Robertson (53). Norén and Nichols have also shown that this body persists through the tetrad divisions of the mother-cell, and Nichols also reported that similar bodies are evident in the later stages of prothallial development. The other archesporial cells occupy positions surrounding the megaspore mother-cell and soon begin to form the tapetal (spongy) tissue.

No special effort was made in the present study to obtain all the stages in the tetrad divisions which produce the megaspores. Norén (47) made a detailed study of this development in *J. communis* and Nichols (45) added confirmatory information to Norén's account. These authors show that in *J. communis* the first division of the mother-cell nucleus gives rise to two nuclei bearing the haploid number of chromosomes. A permanent membrane is rarely, if ever, laid down between these nuclei. The first division thus resembles the first (heterotypic) division of the microspore mother-cell. Usually, only the lower of these two nuclei reaches entirely the resting stage. In this case, only the lower one undergoes the homotypic division, producing a group of three cells, only the lower two of which are true megaspores. Nichols (45) also found certain cases in which the spindles of this homotypic division in *J. communis* appeared similar to and were oriented similarly to the homotypic division in the microspore mother-cell of that species. In *J. virginiana*, only megaspore groups of three cells were ever found (Figs. 83–87), and only in rare instances were these cells seen to form a straight row. Even the middle megaspore of the group in Fig. 85 was somewhat out of line with the top and bottom megaspores. The tetrad divisions were completed by March 26th. Sludsky (62) has reported the presence of two megagametophytes in a single nucellus of *J. communis*, and Nichols (45) found as many as three megaspore mother-cells in tetrad division stages in the same species. No cases like these were observed in *J. virginiana*.

Immediately following the homotypic division, the functional megaspore, which is usually the lowest cell, or at least one of the lower cells, enlarges until it fills the entire space originally occupied by the mother-cell. Meanwhile, the nuclei of the non-functional cells disorganize rapidly (Figs. 86, 87), and are eventually absorbed. A unique condition for conifers in this respect was reported by Lawson (38) for *Pherosphaera*. In this Taxacean genus this author found that three megaspores are formed in an axial row and that all three germinate. The

upper one (viz. the one directed toward the micropylar end) undergoes three nuclear divisions but enlarges very little; the middle one advances to a later free-nucleate stage; while the basal megaspore enlarges more rapidly than the others and ultimately becomes the only one to produce a cellular female gametophyte.

XI. THE FEMALE GAMETOPHYTE

Development of the prothallium.—No special effort was made to obtain the details of the prothallial development in *J. virginiana.* Norén (47) made a thorough study of this process for *J. communis,* and *J. virginiana* agrees in all essentials of this development with Norén's description. Norén showed that the megaspore rapidly gives rise to an embryo sac containing a large central vacuole and a parietal layer of cytoplasm in which are embedded many free nuclei. Nuclear division takes place simultaneously throughout the sac. In the development of the prothallium, open tubes are formed which grow inward toward the center of the embryo sac in the manner first described by Mlle. Sokolowa (63) in 1890. The nucleus of each tube then divides, and cross walls are laid down. The continuation of this process ultimately gives rise to the cellular gametophyte. Nichol's (45) work on *J. communis* was essentially confirmatory of that of Norén in this phase of the life history. In 1900, Arnoldi (3) made a detailed study of the female gametophyte of *Sequoia sempervirens.*

The functional megaspore of *J. virginiana* gives rise to the 2-nucleate female gametophyte early in April. According to Nichols (45), the two nuclei produced by the division of the primary megaspore nucleus in *J. communis* var. *depressa* occupy a central position, and the cell contains several vacuoles. Nichols also observed in the same species that at the 4-nucleate stage these small vacuoles flow together to form one large central vacuole, and the cytoplasm with its included nuclei comes to lie about the periphery of the young embryo sac. By April 15, the young gametophyte of *J. virginiana* has reached its 8-nucleate stage (Fig. 88). Figure 88 shows the young gametophyte to be invested by a layer of loosely associated tapetal cells and a few of the adjacent nucellar cells. The tapetal cells persist in having denser cytoplasts than the nucellar cells until the gametophyte has become cellular. Later stages in the development of the gametophyte of *J. virginiana* are represented in Figs. 89 and 94. As pointed out by Norén (47) for *J. communis,* all the nuclei of the free-nucleate gametophyte of *J. virginiana* divide simultaneously. Figs. 90 and 91 show two stages in

the mitosis of two of these nuclei. Fig. 94 shows an intermediate stage in cell wall formation in the gametophyte.

Nichols (45) states that the volumetric ratio of the gametophyte of *J. communis* just prior to the formation of cellular tissue as compared with the megaspore at the time of its first division is about 12,500:1, and that the embryo sac finally assumes the shape of a prolate spheroid whose longitudinal axis measures about 1400μ. Miss Ferguson (25) found that the full-sized free-nucleate gametophyte of *Pinus Strobus* contains about 2,000 nuclei. These facts cited by Nichols and Miss Ferguson seem to agree quite closely with my observations on *J. virginiana*. Figs. 92 and 93 are diagrams of the nucellus and gametophyte of *J. virginiana* drawn to the same scale so as to show the comparative sizes of the 8-nucleate stage and the full-sized gametophyte just prior to its cell wall formation.

Mlle. Sokolowa (63) and Norén (47) state that no cross walls are formed in the early formation of the cellular gametophyte of *Juniperus* until the tube-like cells meet at the center of the prothallial cavity. Miss Ferguson (25) and Mathews (40) have shown that in *Pinus* cross walls are formed in these cells rather early in the growth of the prothallium, and Nichols (45) shows the latter to be true of *J. communis*. It is undoubtedly true of *J. virginiana* as well (Fig. 94).

Thomson (70) described the megaspore membrane of *J. sabina* and *J. virginiana*, and Norén (47) and Nichols (45) studied the megaspore membrane in *J. communis*. These authors agree that this coat is formed during the free nuclear period of the embryo sac and reaches its highest development at about the time of fertilization. It is about 3μ thick and about uniform in thickness except where it thins out considerably in the archegonial region of the prothallium. The coat is composed of two distinct layers, the fibrillar exosporium and the thicker homogeneous endosporium. Thomson stated that the exosporium is suberized, while the endosporium is largely cellulose. He also showed that the megaspore coat of gymnosperms is strikingly similar to that of the microspore coat.

The tapetum of the female gametophyte of gymnosperms is generally recognized to be homologous with the tapetum of the microsporangium. As Norén (47) and Nichols (45) have shown for *J. communis*, the tapetum of the microsporangium of *J. virginiana* as well as that of the female gametophyte is derived from the non-functional cells of the male and female archesporia, respectively. As the free-nucleate megagametophyte develops, these cells multiply rapidly and continue to invest the

young gametophyte. As stated above, these cells contain a rich supply of cytoplasm and numerous small starch grains. Nichols showed this layer to be still present when the cellular tissue of the gametophyte is being organized (Fig. 94), but subsequently rapidly disorganized.

The archegonium.—Between four and ten cells at the center of the extreme micropylar end of the female gametophyte are from the time of their formation a little larger than the other gametophyte cells and are destined to become the archegonia. Fig. 95 shows the very early differentiation of the archegonia on May 28, approximately one week before fertilization. One of the archegonial initials has already divided to cut off the neck initial cell above from the central cell below, while the other one is undergoing this division. As pointed out by Norén (47), the central cell nucleus is at first very similar in size and appearance to the nuclei of the surrounding gametophyte cells. Again, agreeing with *J. communis*, the group of 4–10 archegonia of *J. virginiana* forms a simple complex with no gametophyte cells between them. Figs. 67, 69, and 101 represent longitudinal sections of three different archegonial complexes. Interesting exceptions from this type of archegonial arrangement have been reported in closely related conifer groups. Lawson (35) shows that the numerous archegonia in the gametophyte of *Sequoia sempervirens* are variously grouped but always oriented with their necks directed to the side of the gametophyte, and he states that the archegonial initials arise from certain cells deep within the gametophyte tissue, rather than peripheral cells. Saxton (58) also reported lateral archegonia in *Actinostrobus pyramidalis*. In *Cunninghamia sinensis*, Miyake (42) showed that the archegonial complex usually has a sterile core of prothallial tissue at the center and that the tapetal or sheath layer of cells invests the outer margin of the archegonial complex.

The archegonia mature rapidly, and as is typical for the conifers bearing archegonial complexes, all the activities in all the archegonia of a complex usually occur about simultaneously. After cutting off the neck initial, the archegonium elongates considerably, and the neck initial cell divides twice to give rise to a tier of four neck cells (Figs. 96, 97). The young elongated archegonium contains a thin, peripheral layer of cytoplasm lining the archegonial wall which encloses a large central vacuole extending nearly the full length of the archegonium. The cytoplasmic layer is thicker at the upper end of the archegonium, and the central cell nucleus is embedded in this upper cytoplasm (Fig. 96).

By the time the archegonia have reached their full length the adjacent gametophyte cells have become organized into a single-layered jacket

or sheath completely investing the archegonial complex excepting the archegonial necks. These cells contain denser cytoplasts than their sister gametophyte cells, but like these gametophyte cells they are usually binucleate (Figs. 102, 111). Norén (47) and Nichols (45) stated that while the nuclear division which gives rise to this binucleate condition may be amitotic, mitotic figures have also been observed in the late history of the jacket cells.

Considerable attention has been paid to the peculiar granular deposits which appear in the archegonial cytoplast shortly before division of the central cell. These deposits together with the numerous cytoplasmic striations which radiate about them present striking aster-like configurations which are certainly a conspicuous feature of the archegonium of *J. virginiana*. Norén (47) and Nichols (45) have assigned to these structures the terms "Strahlungscentren" and "asteroids," respectively. Nichols stated that in *J. communis* "one of these asteroids is invariably situated in close proximity to the nucleus [central cell], and a second one may frequently be seen directly below this, while one or more are present in the lower part of the cell." The same arrangement seems to hold also in *J. virginiana*, but except for the constancy of the presence of one of these bodies just below the central cell nucleus, other asteroids are variously placed in different archegonia, and there may be several of these structures in a single archegonium. Several investigators have reported these structures in different groups of gymnosperms. Coker (16) discovered and described them thoroughly in *Taxodium* and suggested that their presence in the lower part of the long archegonium of *Taxodium*, which, as in *Juniperus*, has a large central vacuole separating the lower portion of the archegonium from the upper portion, may serve to provide "a more definite mechanism for the regulation of the entrance of the plastic material (nutrition) at this end." The asteroid located just below the central cell nucleus has been thought to function in the mitosis of this nucleus. (See Figs. 98, 99, 101.) As a rule, these bodies begin to disappear very rapidly soon after the mitosis of the central cell.

The proteid vacuoles, so common in the developing egg cytoplasm of gymnosperms, were first described by Hofmeister (28). They are rather numerous in *J. virginiana* (Figs. 100, 102, 103). They begin to appear at about the time of the central cell mitosis and to disappear soon after fertilization, but they are never as conspicuous nor as large in *J. virginiana* as in the Abietineae. These proteid vacuoles are generally agreed to be in some way concerned in the nutrition of the egg.

A thorough review of the literature on this subject has been presented by Stopes and Fujii (64).

Division of the Central Cell Nucleus.—Norén (47) and Nichols (45) gave thorough descriptions and demonstrations of the division of the central cell nucleus of *J. communis*, which produces the ventral canal nucleus at the top of the central cell cytoplast and the egg nucleus just below it.

The central cell nucleus of *J. virginiana* divides just two or three days prior to fertilization. All of the cells of an archegonial complex divide approximately simultaneously and very rapidly so that it is very difficult to obtain a complete series of stages of this division. The ventral canal nucleus also disappears very soon after it is formed. During this division, the archegonial cytoplast still contains a fairly large central vacuole. Just prior to the division, this nucleus shows a delicate reticulum with a conspicuous central nucleolus. The nuclear membrane on the side toward the asteroid becomes somewhat wrinkled and as described by Nichols, "pressed or drawn inward" (Fig. 98). Fig. 99 shows the telophase of this division. The chromosomes appear as delicate fibrous bands at the poles of the spindle. In Fig. 100 the division has been completed. The degenerate ventral canal nucleus is located at the top of the egg cytoplast, and the egg nucleus has become organized and considerably enlarged. Several investigators have believed that the spindle fibers for the central cell mitosis arise at the locus of the asteroid below the nucleus, but Nichols (45) was convinced that the asteroid does not contribute to the spindle formation "and its only apparent use in *Juniperus* is to form a support for the free lower pole of the spindle, . . ."

By the time of completion of the central cell mitosis, the central vacuole of the archegonium has vanished, the asteroids are disappearing, and the egg cytoplast begins to become rich in nutritive materials. The archegonial complex is surrounded by a specialized layer of jacket cells which are tapetal in nature (Figs. 101, 102). The eggs in the center of a complex are not, however, in contact with the jacket layer except at their lower ends. Since the cytoplasmic content of the centrally situated egg cells appears very similar to that of the outer eggs, it would seem, as Lawson (37) has suggested for *Libocedrus*, as if the food substances are translocated from egg cell to egg cell in the same way they are transferred from jacket cells to egg cell. Unlike the thick egg membranes of the cycads (Chamberlain, 13) and the Abietineae (Ferguson, 25; Mathews, 40), this membrane of *J. virginiana* is exceedingly

thin at the bottom but somewhat thicker toward the top of the archegonium (Figs. 102, 103).

XII. FERTILIZATION

After the division of the central cell nucleus, the lower daughter nucleus becomes the egg nucleus and rapidly enlarges and moves downward in the egg cytoplasm to a point just above the central vacuole. As noted above, however, very soon after this division, the central vacuole begins to diminish gradually until it soon vanishes entirely from the egg cytoplast. Meanwhile, the rapid acquisition of nutritive materials, accompanied by the formation of numerous proteid vacuoles, causes the egg cytoplasm to become much denser (Fig. 100). The young egg nucleus is ovoid and contains a close reticulum of linin threads on which are arranged irregular deposits of chromatin (Fig. 100). At the time of fertilization the egg nucleus is approximately 60 microns long and 42 microns in diameter.

Fertilization occurs about June 4th at Duke University, Durham, North Carolina. Fertilization stages were found in material from the same tree 2 to 3 days before and 2 to 4 days after June 4th. To sum up a few dates of important stages prior to fertilization: (1) the relatively rapid advance of the pollen tube downward from the upper third of the nucellar tip begins about 6 to 7 days before fertilization; and (2) the tip of the pollen tube enters the archegonial chamber above the archegonial complex about 1 to 3 days later; (3) the division of the body cell occurs about 2 to 4 days after the pollen tube has entered the archegonial chamber; (4) fertilization occurs about 2 to 4 days after the production of two sperm cells by division of the body cell.

Very thorough accounts of the fertilization in *J. communis* have been presented by Norén (46, 47) and Nichols (45), and the descriptions of these authors agree with the facts in *J. virginiana*. Figures 102 and 103 show two stages in fertilization in *J. virginiana*. The egg nucleus at the time of fertilization contains an irregular arrangement of linin threads on which are located very irregularly shaped chromatin deposits. Numerous pseudonucleoli (Norén, 47) are also present in the nucleus. Nichols (45) has shown for *J. communis* that just prior to fertilization the pollen tube membrane is dissolved or ruptured directly above the neck of an archegonium, and one of the male cells is squeezed through the neck to the egg, carrying the broken-down neck cells with it. There is no receptive vacuole in the egg of the investigated species of *Juniperus* as in the Abietineae. Nichols observed that the mem-

brane of the sperm of *J. communis* var. *depressa* is usually cast off before the sperm enters the egg, and he frequently found the remnants of the neck cells and the stalk and pollen tube nuclei of this species lying in the upper part of the egg cytoplasm immediately after fertilization. Unlike the condition in the Abietineae, where only the sperm nucleus farthest in advance in the pollen tube functions in fertilization so that only one egg is fertilized by one pollen tube, in *Juniperus* usually each of the two equal sperm cells effects fertilization in two separate archegonia. The relative sizes of the sperm nucleus of *J. virginiana* compared with that of the egg are about 1:4 as pointed out by Nichols for *J. communis* and not 1:1 as reported by Norén for the same species.

The male nucleus is accompanied or followed by its mantle of cytoplasm and starch as it rapidly approaches and finally comes in contact with the upper side of the egg nucleus (Figs. 102, 103). Coker (16) seems to have been the first investigator especially to emphasize the significance of the great abundance of starch furnished by the male cell in fertilization in a gymnosperm, viz. *Taxodium.* All students of gymnosperms prior to Coker's work seemed to agree that the male nucleus slips from its protoplast as it approaches the egg nucleus and leaves the protoplast behind near the point of entrance. Norén and Nichols described the entrance of the male protoplast to the egg in *Juniperus.* But until the present writing only the male nucleus in angiosperms as well as in other plants and animals has been considered as significant if even functional as a part in the fertilization phenomenon. Coker (15) says for *Taxodium* that "taking the occurrence of starch as an indication of the presence of leucoplasts, we find that most of the plastids of the proembryo of *Taxodium* are furnished by the male cell," since all signs of starch in the egg cytoplasm were negligible. This is certainly true for *J. virginiana.* However, as Coker pointed out, chromatophores or leucoplasts, or *mitochondria,* which require special methods to demonstrate, may well be present in the egg cytoplasm and therefore sooner or later contribute to the plastid contents of the embryo cells, although the quantity of starch granules supplied by the sperm cytoplasm compared to quantity appearing in the proembryonic cells seems to be approximately equal. In the light of a recent paper by Anderson (2) on *Antirrhinum majus* L., etc., who thinks it very likely that cytoplasmic inclusions (including plastids) are transmitted from the pollen tube to the egg during fertilization to combine with similar-appearing inclusions already surrounding the egg nucleus, it might be that male starch and plastids are brought into association with female

inclusions (mitochondria, etc.) during fertilization in *J. virginiana* so that the ultimate embryonic cells may be supplied with a male and a female complement of cytoplasm and cytoplasmic inclusions. As far as the visible starch content of the proembryonic cells of *J. virginiana* is concerned, however, at least its plastids seem to have been entirely furnished by the male cell (Figs. 102-109; 114), and with each mitosis of the proembryo after fertilization, each daughter cell receives a supply of the starch present in the zygote cytoplasm. Norén (47) noted an increase in the starch content in the zygote cytoplasm after fertilization in *J. communis*. There is also an increase in the volumetric proportion of starch at this time in *J. virginiana* compared with that of the starch sheath of the sperm before fertilization. The obvious explanation of this is that each small granule (plastid?) brought in by the sperm cytoplast receives an additional deposit of starch after it enters the egg cytoplast, causing a marked increase in the total volume of starch.

As may be noted in Figs. 102 and 103, a conspicuous vacuolation is left in the egg cytoplasm behind the sperm as it advances to unite with the egg nucleus. This has already been explained by Norén (47) as being due to the sperm's pushing the egg cytoplasm downward and sidewise out of its path. The sperm nucleus usually causes a considerable depression in the egg nucleus (Fig. 103), as has been described for other conifers, and comes to lie in this depression. The sperm nucleus reaches the egg nucleus just above the center of the egg and immediately thereafter both of the nuclei move to the base of the egg cytoplast where they become invested by the starch sheath derived from the sperm cell and their fusion ultimately occurs. Nichols (45) concluded that in *J. communis* dissolution of the sperm and egg nuclear membranes occurs at their points of contact before any indication of the first segmentation division appears.

XIII. EMBRYO FORMATION

Development of the proembryo.—Blackman (5), Chamberlain (12), and many other investigators of conifers have shown that the chromatin substance of the male and female nuclei may be distinguished in the early preparation of the fusion nucleus to divide. Blackman also pointed out that it is only when the "half chromosomes derived from the male and female nuclei respectively fuse together at the poles of the first segmentation spindle" that fertilization may be considered as completed. Norén (47) states that in *J. communis* the chromatin of the male and female nuclei is gradually transformed into pseudonucleoli

which become grouped on either side of the dividing membranes of the fusion (zygote) nucleus. These pseudonucleoli then become arranged into two spiremes and the mitosis proceeds to completion. Nichols (45) describes for *J. communis* var. *depressa* that the zygote nucleus rests for one or two days after fusion and following this there gradually begins to be constructed in the nucleus what appears to be two spiremes of moniliform threads well differentiated into chromatin and linin. These spiremes then draw themselves together and the segmentation division occurs. Fig. 104 shows the metaphase of the first division of the fusion nucleus in *J. virginiana*, and Figs. 105a and 105b represent the resting stage of the first two sporophytic nuclei produced in this division. The chromosomes of the early sporophytic mitoses are strikingly slender curved rods (Figs. 104, 106, 110). The diameter of the fusion nucleus at this time is exceptionally great, and the faint protoplasmic meshwork described by Nichols (45) as being sometimes noticeable in the mature egg nucleus before fertilization is striking at this time (Fig. 104). The fusion nucleus together with the investing starch sheath almost completely fills the base of the archegonial cavity from side to side.

The poles of the mitotic spindle of the fusion nucleus are so arranged that the two resulting groups of chromosomes become located one above the other in the base of the archegonium. After the completion of the first segmentation division, and during the organization of the two resting nuclei, the investing layer of cytoplasm and starch presses in between the two daughter nuclei so that each nucleus becomes surrounded by approximately an equal amount of starch (Figs. 105a, 105b). Thus a 2-nucleate proembryo, or the first two nuclei of the sporophyte, is formed.

The subsequent divisions which give rise to the 12-celled proembryo occur in rapid succession, three days being the maximal time required. The second sporophytic division (Fig. 106) gives rise to 4 free nuclei which arrange themselves in a tetrad at the base of the archegonial cytoplast, each nucleus still being well invested by the starch accumulation (Fig. 107). The third sporophytic division produces eight free nuclei which become variously arranged at the base of the archegonial cytoplasm (Figs. 108, 109); but the usual arrangement seems to be that of three tiers, in which the order from top to bottom is 4, 3, 1 nuclei as in Fig. 109. Wall formation now sets in to cut off each of the eight sporophytic nuclei together with a quantity of cytoplasm and starch, leaving only the four cells of the upper tier open to the archegonial

cytoplasm above. The cytoplasm of these early sporophytic cells shows a marked radiation about the nuclei with cytoplasmic strands extending to and connecting with the limiting plasma membranes (Fig. 109).

Soon after the 8-celled proembryo stage, the four cells of the upper tier divide simultaneously (Fig. 110) to form the 12-celled proembryo, of which only the four cells of the uppermost tier remain open above. These are termed the rosette tier of the proembryo (Fig. 111). This marks the end of the proembryonic development that takes place prior to the elongation of the proembryo suspensors. The proembryonic development up to this point in *Juniperus* agrees with the other Cupressineae and *Pinus* (Ferguson, 25; Mathews, 40) and the other Abietineae. But as shown by Coulter and Chamberlain (19) and Mathews, a further division occurs in the lowest tier of the proembryo of *Pinus* prior to elongation.

Miss Ferguson (25) showed that in *Pinus* all the divisions which occur prior to the establishment of the 12-celled proembryo take place "under the direct influence of the egg-cytoplasm . . . suggesting . . . a closer relationship with those lower gymnosperms in which many free nuclei arise in the egg before the deposition of cell-walls." Immediately following the production of the 12-celled proembryo in *J. virginiana*, the tier of cells just below the open rosette tier begins rapid elongation (Fig. 111). At this time the formerly active jacket cells of the archegonial complex appear considerably elongated, degenerated and crushed. Active tapetal function is now assumed by the gametophyte cells just below the archegonial complex. Iodine stained preparations reveal these cells to contain numerous starch grains, and their cytoplasts are dense and apparently very active. Gradually a sort of "cord" or "core" of such cells becomes distinguishable in a progressive development from below the archegonial complex and extending down the center of the gametophyte.

Origin and Formation of the Embryo.—Hofmeister (28) made a few observations on the embryogeny of the Cupressineae in his investigations of *Thuja occidentalis, Juniperus communis* and *J. virginiana.* Land (32) included a treatment of this subject in his morphological study of *Thuja*; Saxton made a series of studies of the embryogeny of *Tetraclinis* (59), *Widdringtonia* (55, 56), *Callitris* (57) and *Actinostrobus* (58); and more recently Buchholz has made detailed examinations of this phase of development of *Biota* (7) and *Chamaecyparis* (9). Saxton found the embryogeny as well as other phases of the life histories of

Tetraclinis, Widdringtonia, Callitris and *Actinostrobus* so distinctive and different from other investigated Cupressineae that he suggested placing these four genera in a separate tribe of conifers, the Callitrineae, coordinate with the Cupressineae. Hence, except for Land's study of *Thuja* in 1902, embryological investigations of species which are unquestionably Cupressinean had been comparatively meagre until Buchholz's critical examination of *Biota*, etc. (7), in 1926 and *Chamaecyparis obtusa* (9) in 1932. Using a combination of paraffin serial sections and Buchholz's dissection technique (Buchholz, 7), the following is the first detailed account to appear on the later embryogeny of a species of *Juniperus.**

In the conifer proembryo corresponding with that of Fig. 111 of this paper, the group of cells below the elongating so-called suspensor tier of cells seems to be generally regarded as "embryo initial" cells. The subsequent history of these cells does not bear out this idea, unless an "embryo initial" cell may be so flexible a term as to embrace a cell which can give rise to a suspensor initial cell and an embryonic cell. Buchholz showed for *Biota* (7) and *Chamaecyparis* (9) that during the elongation of the first suspensor cells to elongate, which he terms "prosuspensors," and the pushing of these "embryo initials [end cells of proembryo] into the enlarging cavity of the gametophyte," the embryo initials divide at their tips to produce a large cell above and small cell, the embryonic cell, at the end. Then this author states that "almost immediately the larger proximal cells so cut off form suspensors which correspond to the primary suspensor cells in *Pinus*."

In the early proembryo of *Juniperus virginiana*, it is clear that all the eight cells below the rosette tier are elongating cells, and that embryo initials which give rise by mitoses directly to embryo cells, do not exist in the 12-celled proembryo (Figs. 111–117). The first cells of the proembryo to elongate are those of the prosuspensor tier just below the rosette tier, and considerable elongation occurs the next day after the stage shown in Fig. 110 (Fig. 111). As the prosuspensor cells elongate, the bulk of the cytoplasm of each cell becomes collected at the lower ends (i.e. the morphological apical end of the proembryo) of the cell and contains the nucleus while only a slight parietal layer is

*While this article was in galley proof a paper on "A new type of embryogeny in the conifers" by Miss Phyllis L. Cook appeared in the March number of the American Journal of Botany. In this paper she showed that in *Juniperus communis* the peculiar development of the embryo was essentially the same as brought out in the present paper.

left at the top (Fig. 111). Apparently as soon as the proembryo has filled the archegonial cavity and some pressure is exerted at the ends, the cells at the morphological apex ("S" Fig. 112) begin to elongate, and very soon these last cells to elongate become so oriented that the distal ends of the prosuspensor cells become more or less serially adjoined to the proximal ends of the elongating end cells (Figs. 113, 115). The combination of a prosuspensor cell and an elongating end cell may be regarded as a *suspensor unit* even until after the embryo is produced at the anterior end.

As both tiers of suspensor cells continue to elongate, the tips of those of the lower tier present a decidedly haustorial appearance and contain numerous starch grains embedded in the dense cytoplasm at the tip (Fig. 114). These tips push between the gametophyte cells, gradually digesting them and preparing a rather spacious cavity in the gametophyte for the proembryo (Fig. 115). Soon the whole group of suspensor units begins to coil in the same general direction, and meanwhile the suspensor cells may divide one or more times (Figs. 116–119a). Many cells usually become detached from the surrounding gametophytic tissue and adhere to the suspensor cells where they are gradually absorbed.

After the suspensor units have advanced a little more than one-fourth of their final lengths (Figs. 116, 117), the distal cell of each suspensor unit undergoes mitosis (Fig. 118a, 118b), giving rise to the true embryo initial at the tip and a suspensor cell immediately above (the primary suspensor of Buchholz, 7). Figs. 118a, 118b were made from a paraffin microtome section. Figs. 119a and 119b show the whole proembryo and an enlargement of the tip of a suspensor unit (respectively) just after this mitosis. As may be noted from the above figures and subsequent ones, cleavage polyembryony exists in *J. virginiana* as Buchholz has shown for *Biota* and *Chamaecyparis*. After their establishment, the embryo initials remain dormant for a period of 3 to 4 days while the suspensor cells are rapidly elongating. In addition to the further elongation of the suspensor, the further development of the embryo system entails rapid mitoses of the embryo initial cells, and the establishment of a secondary suspensor by at least one of the suspensor units. Many whole proembryos at different stages of development were carefully dissected to determine the number of cells of which the suspensor portion of the single units is composed. Four such units are shown in Figs. 120a–120d. It is clear that in *J. virginiana* (1) the entire suspensor just prior to the formation of the secondary suspensor is composed of 4–7 cells; (2) the cells of the suspensor constitute a

definite unit and are therefore not as loosely associated as Buchholz has shown for *Biota* and *Chamaecyparis*; and (3) there is some evidence of the proliferation of secondary embryos from prosuspensor cells (Figs. 117, 120a, 120d, 129, 130), but this is not as pronounced as Buchholz found in *Biota* and *Chamaecyparis*.

By the time the terminal primary embryo has reached the 8-celled stage (Fig. 122a), the proembryo system has reached about its maximal length, measuring up to 2¾ mm. (in the coiled state), and the entire system has become a much coiled structure, frequently having as many as 8 turns in it.

As in other conifers that show cleavage polyembryony, the primary embryo (terminal) which becomes and remains most advanced in its development becomes also the final mature embryo of the seed in *J. virginiana*, while the remaining primary embryos as well as any secondary embryos that may be proliferated from prosuspensor cells become abortive and are eventually absorbed. Strasburger (63) studied the development of the primary embryo from the embryonic initial cell in *Thuja occidentalis* and *Juniperus* and found the development rather similar. The essentials of this development in *J. virginiana* are in accord with Strasburger's description (his p. 307). Although the early primary embryonic cell-groups (viz. before secondary suspensor formation occurs) may frequently be formed in various ways (Figs. 120d, 123, 124, 125), usually the embryonic initial cell (Fig. 119b, etc.) divides by a transverse wall which establishes two tiers in the embryonic group (Fig. 121). The lower (end) cell gives rise to only embryonic cells, while at least some of the cells produced by mitoses of the upper member of this pair give rise to the secondary suspensor to be described later. Each of these cells now undergoes numerous mitoses in rapid succession, the first mitosis in the upper cell being longitudinal but transverse in the lower cell. Thus a more or less two-tiered embryonic group of about 12 cells is formed as shown in Fig. 123. The primary suspensor cell bearing this group has become very large, often 55 microns in diameter, and contains a large nucleus near its lower end. All the suspensor cells now contain a scanty supply of cytoplasm.

Buchholz states that the early multicellular embryos of *Biota* (7) and *Chamaecyparis* (9) seem to develop at least for a brief period by apical cell growth as was also noted by Strasburger (66) for *Thuja occidentalis*. I was unable to detect this method of development in the embryo of *J. virginiana*. There were, however, some indications

of fragmentation of the young multicellular embryos of *J. virginiana* (Figs. 120d, 126a), thereby resulting in a secondary cleavage stage in the embryo, as Buchholz found to be a not uncommon characteristic in *Biota*; but *J. virginiana* agrees more with *Chamaecyparis* (Buchholz, 9) in that such a condition is very rare.

By the time the embryonic cell mass has reached a stage of approximately 125–150 cells, the upper (proximal) cells of the cell group begin to elongate posteriorly from the embryo and apply themselves closely to the primary suspensor in the manner described by Buchholz (9) for *Chamaecyparis obtusa*. (See my Figs. 126a, 126b.) These cells continue to elongate and to divide until they produce the massive secondary suspensor system of Buchholz (7, 9). (My Figs. 127–130.) In the backward growth of the secondary suspensor system, the old primary suspensor is observed to become detached and gradually displaced by these elongating suspensors (Figs. 127, 128). Meanwhile the entire primary suspensor system, including all primary embryos except the anterior functional one, begins to collapse and degenerate and will finally be entirely absorbed by the time the functional embryo is mature (Figs. 128–130).

While the secondary suspensor system is developing, the mass of polyhedral embryonic cells at the distal end of the embryo system undergoes rapid mitoses, and throughout at least the majority of the embryonic development, all the observable secondary suspensor units are seen to be in series with the early established vertical rows of embryonic cells below (Figs. 128, 129).

For a considerable period there is no indication of organal differentiation of the embryo, but by July 20 and before the embryo is half grown, the dome-shaped meristematic bud of the stem tip (plumule) becomes recognizable (Fig. 129). The two broad cotyledonary primordia appear about the same time as the plumule. Buchholz (6) showed that the stem tip meristem of *Pinus* is visible prior to the appearance of the cotyledonary primordia, but the reverse is true in *Sciadopitys* (Buchholz, 8) in which species this author stated that "the stem tip is probably delayed until after the seed begins to germinate." The plerome apex of the root in the embryo of *J. virginiana* is recognizable before the plumule and cotyledonary primordia, as Buchholz has also shown for *Pinus* and *Sciadopitys*. The embryo of *J. virginiana* reaches maturity by the last of July, a little over seven weeks after fertilization (Fig. 130). The principal change that occurs from the first appearance of the plumule and cotyledonary primordia until

maturity is a general elongation of the cotyledons and hypocotyl, since the embryo has attained approximately its maximal diameter by the time these organs first appear. A gradual reduction and absorption of the old primary suspensor system has taken place by this time so that very little, if any, of it exists, although a rather elaborate secondary suspensor still remains attached to the upper margin of the root cap. The root cap agrees with *Sciadopitys* in which, according to Buchholz (8), the root-cap embraces less than one-fourth of the embryo, and not with *Pinus*, whose root-cap, according to the same author (6), includes nearly half of the embryonic axis. In the median longitudinal section of the mature embryo of *J. virginiana* (Fig. 130), the approximate arrangement of the cell layers is shown. There are about seven distinct longitudinal layers from the median stele structure to the margin of the embryo, and about 17 layers comprise the dome-shaped root-cap. Fig. 79 shows the proper proportions and relationships of all the tissues which surround the embryo in the mature berry-cone at this time.

XIV. EMBRYOGENY OF THE CUPRESSINEAE AND RÈLATED GROUPS

In reviewing the investigations of the embryogeny of the Cupressineae and closely related groups, there seem to be at least two types known for the Cupressineae, exclusive of those four genera which have been considered as comprising the Callitrineae (see p. 43 above). Strasburger (66), Land (32) and Buchholz (9) have described the production of only a single embryo from the fertilized egg of *Thuja* (simple polyembryony). On the other hand, cleavage polyembryony, or the production of several embryos by a single fertilized egg, has been reported for *Biota* (Buchholz, 7), *Chamaecyparis* (Buchholz, 9), *Libocedrus* (Lawson, 37), and *Juniperus* (see present paper). It should be noted here that several investigators of the Taxodineae (notably Coker (16) on *Taxodium*, Lawson (36) and Buchholz (10) on *Crytomeria japonica*, and Buchholz (8) on *Sciadopitys*) have reported cleavage polyembryony in this subfamily and proembryos similar to those of the Cupressineae. However, Buchholz (9) points out in his paper on *Chamaecyparis* that "it appears that the Taxodineae usually have no primary suspensor" but "have prosuspensors which are morphologically equivalent to the structures so-named in *Chamaecyparis*, on the ends of which the embryo initials develop into multicellular embryos without the intervention of the very long primary suspensor." Saxton's investigations of the embryogeny of

Tetraclinis, *Widdringtonia*, *Callitris*, and *Actinostrobus* (see above), have shown that the archegonia of these genera occupy a lateral position in the female gametophyte and that wall formation in the proembryo occurs at an earlier stage (i.e., as early as during the transition from the binucleate to the four-nucleate condition in *Actinostrobus* and *Callitris*). Saxton reports that the proembryo completely fills the archegonium in these genera, and he shows diagrammatically for *Actinostrobus* that just prior to cutting off "embryo initials" the proembryo is composed of six cells, an apical tier of two cells separated by a wall longitudinal to the long axis of the proembryo and four cells beneath this tier separated by transverse walls. The suspensors elongate laterally from the proembryo but toward the base of the gametophyte, since the proembryo itself is laterally located in the gametophyte. The zygotes all have cleavage polyembryony as is true of the other investigated Cupressineae except *Thuja*.

XV. SUMMARY

Juniperus virginiana L. is a dioecious conifer native to eastern United States and southern Ontario. This paper is a study of the morphological and cytological development of the sporophylls and seed of this species.

The staminate cones begin to develop in August and attain their mature size by winter. Three to four microsporangia develop as marginal evaginations at the base and on the lower side of the microsporophyll; and immediately distal to the microsporangia, the downward development of a broad, flattened outgrowth from the microsporophyll completes the formation of the sporophyll's peltate shape. The large resin cavity of the microsporophyll originates schizogenously.

By late September the pollen grains are formed and contain an abundance of large starch grains. By the time the exine of the mature pollen grain is complete the following spring, this starch supply has disappeared. The mature sporangial wall consists of a single layer of secondarily thickened cells.

Meiosis of the pollen mother-cells resembles that of other conifers. The mother-cell nucleus is about one-third as large as that of *Larix* and *Pinus*, and the reduced number of chromosomes is eleven. At meiosis, the mother-cell cytoplasm presents a strikingly alveolar appearance due to its starch content, and at completion of the first

meiotic division, starch grains show an alternate arrangement on either side of the protoplasmic layer midway between the two resting daughter nuclei.

No further division occurs in the pollen grain until after pollination.

No sterile prothallial cells are formed, and the mature pollen grain is wingless.

Pollination occurs between the middle and last of February. The internal cellulose bands of the microsporangial wall cells facilitate sporangial rupture in a manner comparable to the thickened portions of the annular cells of leptosporangiate ferns. The pollination-drop is particularly noticeable in *J. virginiana* at the time of pollination.

The division giving rise to the generative cell and the tube cell resembles that of *Taxodium* but occurs after pollination. The pollen grain germinates soon afterwards; the generative cell divides to form the body cell and stalk cell nucleus about three weeks later, by which time the pollen tube has grown some distance at the tip of the nucellus.

As in other conifers, the early growth of the pollen tube is slow and considerably branched in the nucellar tip. About the last of May, however, it shows a much more active and direct growth, and within a very few days penetrates to the necks of the archegonia.

The mature body cell possesses a dense cytoplast which contains a rich starch supply as is characteristic of Cupressineae, except possibly *Widdringtonia* (Saxton, 56). This cell divides about three days before fertilization (i.e., about June 1st) and simultaneously with the central cells of the archegonial complex. The resulting two male cells are equal and have contents and a thick membrane similar to the body cell.

The ovulate cones begin their development about the first of September and continue slight growth during the winter. They are borne terminally on axillary branches of the current season. Usually two pairs of megasporophylls, pairs *a* and *b* from summit of cone-axis downward, are borne decussately on the short cone-axis, but sometimes a lower pair or a rather rudimentary apical pair may enter into cone formation. Only sporophyll pair *b* is fertile. In approximately two-thirds of the female flowers, only one sporophyll is fertile and bears a single ovule, and in about half of the remaining flowers the one fertile member of pair *b* bears two ovules, while both members of pair *b* of the remaining flowers are fertile, each bearing one ovule. The ovules appear about the middle of September and are borne in the

axil of the fertile megasporophylls. An outgrowth, the fruit-scale, develops on the morphological upper side of the megasporophylls. Like the more distally located cover-scale of the sporophyll, the fruit-scale soon becomes supplied with a separate vascular bundle. The fruit-scales of the four megasporophylls continue to grow upward and finally coalesce above the ovule or ovules to produce approximately the upper half of the outer fleshy coat of the small, blue berry-like cone.

The mature berry-cone of *J. virginiana* has a stony integumentary layer which forms a protective coat entirely surrounding the seed. The secondary thickening of the walls of the cells which produce this layer begins developing about May 1st in the micropyle-closing layer and progresses downward through the middle region of the integument until the completion of the lignification process about June 6th. The thick-walled stone cells of this layer bear for the most part long and narrow simple pits.

While the ovules appear about the middle of September, the megasporophylls are not recognizable to the naked eye until about the time of pollination the following spring. During pollination, the single megaspore mother-cell may be distinguished near the base of the nucellus. Reduction division gives rise to two nuclei, only the lower of which divides equationally. The three resulting cells do not usually lie in a straight row but are more frequently grouped in a manner resembling the tetrad arrangement of the microspores. One of the two lower megaspores resulting from the second division develops into the female gametophyte, the other two cells disorganizing.

The archegonia, usually four to ten in number, are arranged in a close complex at the center of the extreme micropylar end of the female gametophyte as in other Cupressineae. The complex is surrounded closely by a tapetal layer which is more densely supplied with cytoplasm than its surrounding sister megagametophytic cells. Each archegonium has a tier of four neck cells. The central cell is long and contains several "asteroids" in its cytoplasm. An extremely short-lived ventral canal nucleus is cut off rapidly and simultaneously in all the central cells of the archeogonial complex about three days before fertilization.

Fertilization occurs from about June 1st to 7th at Chapel Hill, N. C. Usually the two equal male cells of a pollen tube effect fertilization in two archegonia. The male nucleus is somewhat smaller than the

egg nucleus. As in other Cupressineae, as well as in Taxodineae, the starch-filled male cytoplast accompanies the nucleus in fertilization, passes through the cytoplasm at the tip of the egg and during fusion of the two sexual nuclei entirely enfolds them. This starch and cytoplasm also accompany the fusion nucleus when it subsequently passes to the base of the archegonium and are more or less equally distributed to the cells of the proembryo.

Resulting from three divisions of the fusion nucleus, eight free nuclei are formed at the base of the archegonium and become variously arranged but usually so that one nucleus is lowermost. Cell walls are now formed but the upper tier of nuclei, usually four, is left open. This tier now divides by walls at right angles to the long axis of the archegonium, producing the rosette of free nuclei above and the so-called suspensor tier immediately below.

In the twelve-celled proembryo of *J. virginiana,* all the eight cells below the rosette tier elongate, the upper tier, however, elongating first. Cleavage polyembryony is assured early, since the tips of the morphologically anterior suspensor cells are separated early by their varied directions of penetration of the megagametophyte. Soon, however, a cavity is digested in the gametophyte allowing the suspensor units to become more closely associated. After the suspensor units of a whole proembryo have grown about a fourth of their final length (i.e., about .6 mm.) and each unit is composed of two to three suspensor cells, the anterior cell of each unit divides to produce an apical embryo initial cell and the primary suspensor cell. The whole suspensor portion of the proembryo continues to elongate in a spiral fashion, sometimes reaching the ultimate length of 2.75 mm. and showing seven to eight turns in its spiral structure. Meanwhile, the suspensor cells continue to divide, and each suspensor unit is often composed of four to seven cells.

By the continued division of the embryo initial cell at the tip of each suspensor unit, a small group of embryonic cells is formed by the time the suspensor units have completed their elongation. This embryonic group can sometimes be seen to be somewhat constricted into two tiers of cells. The upper cells of the upper tier soon begin to elongate posteriorly to produce a very extensive secondary suspensor system as in *Biota* and *Chamaecyparis*, while the anterior tier of cells contributes most to formation of the embryo proper. Thus the embryo initial of each suspensor unit of the proembryo gives rise to a separate embryo thereby producing several embryos from a

single archegonium. Usually the developing embryo which is most anterior becomes larger than the rest and produces the final single embryo of the seed.

The mature embryo is dicotyledonous and reaches maturity about the last of July.

ACKNOWLEDGMENTS

Grateful acknowledgment is made of the invaluable aid rendered during the course of this investigation by Dr. W. C. Coker under whose immediate supervision the work was carried on. The writer also wishes to acknowledge his indebtedness to Dr. H. R. Totten for the opportunity of comparing materials and slides with those prepared by him; to Dr. John N. Couch for helpful advice with details; and to Dr. J. E. Adams for valuable assistance throughout this investigation, especially with its morphological phases. Thanks are also extended to Dr. H. L. Blomquist and Dr. F. A. Wolf of Duke University for assistance at the beginning of the study and to Miss Alma Holland for assistance in the preparation of the plates.

XVI. BIBLIOGRAPHY

1. ALLEN, C. E. 1905. Nuclear division in the pollen mother-cells of *Lilium canadense*. Ann. Bot. **19**: 189-258.
2. ANDERSON, L. E. 1936. Mitochondria in the life cycles of certain higher plants. Amer. Jour. Bot. **23**: 490-500.
3. ARNOLDI, W. 1900. Beiträge zur Morphologie einiger Gymnospermen. I. Die Entwicklung des Endosperms bei *Sequoia sempervirens*. Bull. des Natur. de Moscou. Nos. 2 & 3. pp. 1-13.
4. BELAJEFF, WL. 1893. Zur Lehre von dem Pollenschlauche der Gymnospermen. Ber. Deut. Bot. Gesell. **11**: 196-201.
5. BLACKMAN, V. H. 1898. On the cytological features of fertilization and related phenomena in *Pinus silvestris*. Phil. Trans. Roy. Soc., Series B, **190**: 395-426.
6. BUCHHOLZ, J. T. 1918. Suspensor and early embryo of *Pinus*. Bot. Gaz. **66**: 185-228.
7. ——— 1926. The embryogeny of the conifers. Proc. of Intern. Cong. of Plant Sci. **1**: 359-392.
8. ——— 1931. The suspensor of *Sciadopitys*. Bot. Gaz. **92**: 243-262.
9. ——— 1932. The embryogeny of *Chamaecyparis obtusa*. Amer. Jour. Bot. **19**: 230-238.
10. ——— 1932. The suspensor of *Cryptomeria japonica*. Bot. Gaz. **93**: 221-226.
11. CALDWELL, O. W. 1907. *Microcycas calocoma*. Bot. Gaz. **44**: 118-141.
12. CHAMBERLAIN, C. J. 1899. Oogenesis in *Pinus laricio*. Bot. Gaz. **27**: 268-280.

13. ——— 1906. The ovule and female gametophyte of *Dioon*. Bot. Gaz. **42**: 321–358.
14. ——— 1907. Preliminary note on *Ceratozamia*. Bot. Gaz. **43**: 137.
15. ——— 1933. Methods in Plant Histology. Univ. of Chicago Press, Chicago.
16. COKER, W. C. 1903. On the gametophytes and embryo of *Taxodium*. Bot. Gaz. **36**: 1–27; 114–140.
17. ——— 1904. On the spores of certain Coniferae. Bot. Gaz. **38**: 206–213.
18. COULTER, J. M., AND CHAMBERLAIN, C. J. 1901. Morphology of Spermatophytes. D. Appleton & Co., New York.
19. ——— 1917. Morphology of Gymnosperms. Univ. of Chicago Press, Chicago.
20. ——— 1932. Morphology of Gymnosperms. Univ. of Chicago Press, Chicago.
21. COULTER, J. M., and LAND, W. J. G. 1905. Gametophytes and embryo of *Torreya taxifolia*. Bot. Gaz. **39**: 161–178.
22. DLUHOSCH, H. 1937. Die Blüten der Coniferen: Teil III. Entwicklungsgeschichte Untersuchungen über die Mikrosporophyllgestaltung der Coniferen. Bibliotheca Botanica **28**: 1–24.
23. DOAK, C. C. 1932. Multiple male cells in *Cupressus arizonica*. Bot. Gaz. **94**: 168–182.
24. DOYLE, J., AND CLINCH, P. 1927. Seasonal changes in conifer leaves, with special reference to enzymes and starch formation. Proc. Roy. Irish Acad. Sect. B. **37**: 373–414.
25. FERGUSON, MARGARET C. 1904. Contributions to the knowledge of the life history of *Pinus* with special reference to sporogenesis, the development of the gametophytes and fertilization. Proc. Wash. Acad. Sci. **6**: 1–202.
26. HABERLANDT, G. 1928. Physiological Plant Anatomy. MacMillan & Co., London.
27. HIRMER, M. 1936. Entwicklungsgeschichte und vergleichende Morphologie des weiblichen Blütenzapfens der Koniferen. Teil I. Bibliotheca Botanica, H. 114, 100 S., 31 Texttabb., 12 Taf., 11 Tabellen.
28. HOFMEISTER, W. 1851. Vergleichende Untersuchungen höherer Kryptogamen und der Samenbildung der Coniferen. Leipzig.
29. ——— 1858. Neuere Beobachtungen über Embryobildung der Phanerogamen. Jahrb. für Wiss. Bot. **1**: 82–188, Taf. 7–10.
30. JACK, J. G. 1893. The fructification of *Juniperus communis*. Bot. Gaz. **18**: 369–375.
31. JUEL, H. O. 1904. Uber den Pollenschlauch von *Cupressus*. Flora **93**: 56–62.
32. LAND, W. J. G. 1902. A morphological study of *Thuja*. Bot. Gaz. **34**: 249–259.
33. LANFER, K. F. 1933. Ontogenetische und teratologische Untersuchungen über die weiblichen Blütenzapfen der Abietineen, der Cupressaceen und der Taxaceen mit besondere Berücksichtigung der bei einigen Arten vorkommenden Vergrünungen (Anamorphosen). Ein Beitrag zur Kenntnis der weiblichen Blüten der Coniferen. Engler: Bot.

Jarhb. für Systematik, Pflanzengeschichte und Pflanzengeographie **65**: 508–551.
34. ——— 1934. Beitrag zur Klärung und zum richtigen Verständnis der organogenetischen Untersuchungen der Coniferen-zapfen, von O. Hagerup. Engler: Bot. Jahrb. **66**: 471–480.
35. LAWSON, A. A. 1904. The gametophytes, archegonia, fertilization and embryo of *Sequoia sempervirens*. Ann. Bot. **18**: 1–28.
36. ——— 1904. The gametophytes, fertilization and embryo of *Cryptomeria japonica*. Ann. Bot. **18**: 417–444.
37. ——— 1907. The gametophytes and embryo of the Cupressineae with special reference to *Libocedrus decurrens*. Ann. Bot. **21**: 281–301.
38. ——— 1923. The life-history of *Pherosphaera*. Proc. of Linn. Soc. of New South Wales **48**: 499–516.
39. MAHESHWARI, P. 1937. A critical review of the types of embryo-sacs in Angiosperms. New Phytol. **36**: 359–417.
40. MATHEWS, A. C. 1932. The seed development in *Pinus palustris*. Jour. of Eli. Mitch. Sci. Soc. **48**: 101–118.
41. MIRBEL, DE, AND SPACH. 1843. Notes sur l'embryogenie des *Pinus Laricio* et *sylvestris*, des *Thuja orientalis* et *occidentalis*, et *Taxus baccata*. Ann. Sci. Nat. Bot., 2 serie, **20**: 257–265.
42. MIYAKE, K. 1910. The development of the gametophytes and embryogeny in *Cunninghamia sinensis*. Beih. zum Bot. Centralb. **27**: 1–25.
43. MOHR, CHARLES. 1901. Notes on the red cedar. U. S. Dept. of Agri. Div. of Forestry. Bulletin No. 31, pp. 1–37.
44. MOTTIER, D. M. 1907. The development of the heterotypic chromosomes in pollen mother cells. Ann. Bot. **21**: 309–347.
45. NICHOLS, GEORGE E. 1910. A morphological study of *Juniperus communis* var. *depressa*. Beih. Bot. Centralb. **25**: 201–241.
46. NORÉN, C. O. 1904. Über die Befruchtung bei *Juniperus communis*. Vorläufige Mitteilung. Archiv. Bot. k. Svensk. Vetensk-Akad. **3**: No. 11, pp. 1–11.
47. ——— 1907. Zur Entwicklungsgeschichte des *Juniperus communis*. Uppsala Univ. Arsskr., pp. 1–64.
48. OTTLEY, ALICE M. 1909. The development of the gametophytes and fertilization in *Juniperus communis* and *Juniperus virginiana*. Bot. Gaz. **48**: 31–46.
49. PILGER, R. 1926. Engler-Prantl: Die Natürlichen Pflanzenfamilien **13**: 121–403. Leipzig.
50. ——— 1934. Vergleichende Studien der weibliche Coniferenblüten. Engler: Bot. Jahrb. **66**: 469–471.
51. PROPACH-GIESELER, CHARLOTTE. 1936. Die Blüten der Coniferen. Teil II: Zur Morphologie und Entwicklungsgeschichte der weiblichen Blütenzapfen der Cupressaceen. Bibliotheca Botanica **114**: L. 2.
52. QUISUMBING, EDUARDO. 1925. Stony layer in seeds of gymnosperms. Bot. Gaz. **79**: 121–195.
53. ROBERTSON, A. 1904. Spore formation in *Torreya californica*. New Phytol. **3**: 133–148.

54. SAX, K., AND SAX, HALLY J. 1933. Chromosome number and morphology in the conifers. Jour. Arnold Arboretum **14**: 356–375.
55. SAXTON, W. T. 1909. Preliminary account of the ovule, gametophytes, and embryo of *Widdringtonia cupressoides*. Bot. Gaz. **48**: 161–178.
56. ——— 1910. Contributions to the life history of *Widdringtonia cupressoides*. Bot. Gaz. **50**: 31–48.
57. ——— 1910. Contributions to the life history of *Callitris*. Ann. Bot. **24**: 557–569.
58. ——— 1913. Contributions to the life history of *Actinostrobus pyramidalis*, Miq. Ann. Bot. **27**: 321–345.
59. ——— 1913. Contributions to the life history of *Tetraclinis articulata*, Masters, with some notes on the phylogeny of Cupressineae and Callitroideae. Ann. Bot. **27**: 577–603.
60. SCHNARF, K. 1933. Handbuch der Pflanzenanatomie. II. Abt. 2 Teil. Embryologie der Gymnospermen. Verlag von Gebrunder Borntraeger. Berlin.
61. SHARP, L. W. 1926. An Introduction to Cytology, 2nd Edition. McGraw-Hill Book Co., New York.
62. SLUDSKY, N. 1905. Über die Entwicklungsgeschichte des *Juniperus communis*. Vorläufige Mitteilung. Ber. Deutsch. Bot. Gesell. **23**: 212–216.
63. SOKOLOWA, C. 1890. Naissance de l'endosperme dans le sac embryonnaire de quelques Gymnosperms. Bull. de la Soc. des Nat. de Moscou, No. 3, p. 446.
64. STOPES, M. C., AND FUJII, K. 1906. The nutritive relations of the surrounding tissues to the archegonia in gymnosperms. Beih. Bot. Centralb. Abt. 1, **20**: 1–24.
65. STRASBURGER, E. 1869. Die Befruchtung bei den Coniferen. Jena.
66. ——— 1872. Die Coniferen und die Gnetaceen. Jena.
67. ——— 1878. Ueber Befruchtung und Zelltheilung. Jena.
68. ——— 1879. Die Angiospermen und die Gymnospermen. Jena.
69. ——— 1892. Ueber das Verhalten des Pollens und die Befruchtungsvorgänge bei den Gymnospermen. Histologische Beiträge, part 4, pp. 1–46.
70. THOMSON, R. B. 1905. The megaspore-membrane of the Gymnosperms. Univ. Toronto Studies, Biol. Ser., No. 4. Published by the librarian.

XVII. EXPLANATION OF PLATES

PLATE 1

Fig. 1. Longitudinal section of male branch and bud before male cone is recognizable. r.c., resin cavity. × 17. Aug. 8.

Fig. 2. Longitudinal section of male cone in earliest stages of microsporangial origins. × 17. Aug. 8.

Fig. 3. Longitudinal section of a nearly mature male cone. sg., sporangium. × 17. Sept. 23.

Fig. 4. Mature male cone. × 3.

Fig. 5. Longitudinal section of microsporophyll before sporangial formation sets in. ch.c., chlorophyll-bearing cells; r.c., resin cavity; r.ch., resin channel; r.cl., resin cell. × 110. Aug. 8.

Fig. 6. Longitudinal section of microsporophyll showing very early stage in sporangial formation. r., resin. A plate of three archesporial cells is present here. × 110. July 28.

Fig. 7. Later stage in sporangial formation. Arrow indicates mid-line of cone axis. × 110. July 28.

Fig. 8. Longitudinal section of portion of microsporophyll showing young sporangium with central primary sporogenous tissue surrounded by one-layered tapetum and the two-layered sporangial wall. r.c., resin cavity. × 110. July 28.

Fig. 9. Microsporangium with mature pollen mother-cells. p.m.c., pollen mother-cell; t., tapetum. × 110. Sept. 23.

Fig. 10. Fate of the enveloping walls of microsporangium. Inner sporangial wall layer degenerating. t., tapetum. × 490. Sept. 23.

Fig. 11. Microsporangial wall layers and tapetum after young pollen grains are developed. The pollen grains are richly supplied with starch. × 490. Sept. 23.

Fig. 12. Longitudinal section of mature microsporophyll before pollination. The outer sporangial wall layer alone persists. p.g., pollen grain; r.cl., resin cell. × 56. Feb. 12.

PLATE 2

Fig. 13. Microsporangial wall cells. Longitudinal view. n., nucleus. × 552. Feb. 12.

Fig. 14. Microsporangial wall cells. Cross section view; somewhat diagrammatic. × 552. Feb. 22.

FIG. 15. Fully mature pollen mother-cell. × 1200. Sept. 17.

Fig. 16 (1). Mother-cell nucleus approaching synizesis. Pairing of the chromatin material is taking place,—zygonema stage. × 1200. Aug. 21.

Fig. 16 (1)a. Portion of the chromatin structure of Fig. 16 (1). × 1200.

Fig. 16 (2). Synizesis. × 1200. Aug. 21.

Fig. 17. Nucleus recovering from synizesis. Open spireme beginning to form × 1200. Aug. 21.

Fig. 18. Open spireme, uniformly distributed. Alveolar (or frothy) appearance of cytoplasm from here on is due largely to the accumulation of a rich starch content. × 1200. Aug. 21.

Fig. 19. Pachynema stage. Obviously paired chromatin material shows transverse segmentation. × 1700. Sept. 23.

Figs. 20, 21. Strepsinema stages. The chromatin segments are shortening, thickening and somewhat twisting. × 1200. Aug. 21.

Fig. 22. Near diakinesis stage. Chromosomes have attained compact form just prior to formation of the spindle. × 1200. Aug. 21.

Figs. 23, 24. Meta- and telophases of the heterotypic division. × 1200. Aug. 21.

Fig. 25. Resting daughter nuclei of the heterotypic division. The characteristic cytoplasmic configuration midway between the nuclei is due to thicker cytoplasm here and a sort of alternate arrangement of large starch grains on either side of it. × 1200. Sept. 23.

Fig. 26. Polar view of resting dyad nucleus. × 1200. Sept. 23.

Fig. 27. Telophase of homotypic division. × 1200. Sept. 23.

Fig. 28. Cell plates being formed between the cells of the young tetrad. The cytoplasm is rich in starch. × 1200. Sept. 23.

Figs. 29, 30. Polar views of stages as in Fig. 28, showing the haploid number of chromosomes to be eleven. Fig. 29, × 1700; Fig. 30, × 1200. Sept. 23.

Fig. 31. Young tetrad of microspores surrounded by pollen mother-cell wall. × 1200. Sept. 23.

Fig. 32. Microspores within thick-walled chambers of tetrad. × 1200. Sept. 23.

Fig. 33. Microspores being liberated from tetrad chambers. × 1200. Sept. 23.

Fig. 34. Young microspore after being freed from tetrad chamber. × 1200. Sept. 23.

Fig. 35. Intine and exine walls developing around pollen grain. × 1200.

Fig. 36. Mature pollen grain with cytoplasm rich in starch in resting condition prior to the winter rest period. × 1200. Sept. 23.

Fig. 37. Pollen grain just before pollination. Very little, if any, starch is now present. × 1200. Feb. 12.

Figs. 38–40. Pollen mother-cells and a microspore dyad showing reaction to starch test in different stages of development. × 1200. Aug. 21.

Fig. 41. Pollen grain prior to germination at tip of nucellus. nuc., nucellus. × 1200. Feb. 25.

Figs. 42, 43. Early germination of pollen grain at tip of nucellus. The mucilaginous pollination-drop is present at the tip of the nucellus at this time. g.c., generative cell; p.t.n., pollen tube nucleus; p.d., pollination-drop. × 1200. Mar. 22 and 26, resp.

PLATE 3

Fig. 44. Pollen tube penetrating tip of nucellus. p.t.n., pollen tube nucleus. Generative cell (g.c.) has changed from lenticular to spherical shape. × 490. Mar. 30.

Fig. 45. Pollen tube and contents after division of generative cell, the whole still near tip of nucellus. b.c., body cell; st.n., stalk cell nucleus; p.t.n., pollen tube nucleus. × 490. Apr. 19.

Fig. 46. Pollen tube and contents. The body cell is acquiring a densely granular cytoplast. b.c., body cell. × 490. May 19.

Figs. 47–49. About the time of fertilization. (See below.)

Fig. 47. Just prior to division of body cell (b.c.) and subsequent to division of central cell. The body cell cytoplast is filled with starch granules. The egg nucleus has not attained yet its full size. eg., egg cell. × 234. June 4.

Fig. 48. After division of body cell to form two equal sperm cells (sp.). × 234. June 3.

Fig. 49. Median section of a mature sperm cell and edge of its partner, the latter shown in outline. × 530. June 3.

Fig. 50a. Young female branch when cone branches are first recognizable. Branches marked "X" bear very young ovules at their tips. × 5. Sept. 16.

Fig. 50b. Ovule from a branch "X" of Fig. 50a. nuc., nucellus. × 56.

Fig. 51. Most common type of female flower, showing three opposing pairs of sporophylls and one ovule. From the tip of the floral branch, they run in order a, b, c. One member of pair "b" is fertile. The fertile sporophylls are underlined. × 8. About Mar. 29.

Fig. 52. An uncommon flower type with an additional pair of sporophylls, a′, which is sterile and above pair a on the cone axis. × 8. About Mar. 29.

Figs. 53a, 53b. A not uncommon flower type with one of the members of pair b being fertile and bearing two ovules. Contrary to the one-ovule type, in the two-ovule type, the sporophyll pair c usually enters into the sporphyll fusion to produce the berry-cone. × 8. About Mar. 29.

Fig. 54. Two-ovule flower type in which one ovule is borne by each member of sporophyll pair b. × 8. About Apr. 5.

Figs. 55–59. Female floral diagrams to show the most common floral configurations. The sporophyll pairs which usually enter into the berry-cone formation are shaded in, and the order from cone apex downward is labeled from a′ and a, etc. ov., ovule. × 24 and × 44.

PLATE 4

Fig. 60a. Mature one-seeded berry-cone in side view showing cover scale tips, the fertile member of pair b (underlined) being solitary. The seed is outlined. × about 4. Jan.

Figs. 60b, 60c. Views at right angle to that of Fig. 60a.

Fig. 61. Mature two-seeded berry-cone in side view. In this type, only one member of sporophyll pair b is bearing the two seeds. × about 2. Jan.

Fig. 62. Mature two-seeded berry-cone in side view, in which each member of sporophyll pair b is bearing one seed. × about 2. Jan.

Fig. 63. Longitudinal section view of female flower just after pollination. Indication of the sporophyll-outgrowth (ovuliferous- or fruit-scale) may be noted here. p.g., pollen grain; m.m.c., megaspore-mother-cell. × 17. Mar. 5.

Fig. 64. Later stage of cone development. The sporophyll outgrowths are advancing above the necks of the ovules, and their separate vascular supplies may be noted. o.s., fruit- (ovuliferous) scale; c.s., cover-scale. × 17. Mar. 30.

Fig. 65. After fusion of the sporophyll-outgrowths above necks of ovules. × 17. Apr. 15.

Fig. 66. Female cone at the beginning of secondary thickening of the integument to form the stony layer of the seed. s.t., stony tissue; m-g., megagametophyte. × 17. May 9.

Fig. 67. Berry-cone showing further progress in stony tissue formation. p.t., pollen tube; s.t., stony tissue; nuc., nucellus; ar., archegonium; m-g., megagametophyte. × 17. May 26.

Fig. 68. Longitudinal section showing method of closure of sporophyll-outgrowths at tip of berry-cone. cu., cuticle. × 110. About May 26.

Fig. 69. Seed in longitudinal section view just after fertilization to show diagrammatically the nearly final stage in the stony layer development. × 17. June 6.

Fig. 70. Tip of integument in longitudinal section view to show details of beginning of stony tissue formation. Cell "X" is about the first cell below the micropyle-closing cells (mi. cl.) to show the secondary thickening. × 234. May 1.

Fig. 71a. Slightly later stage in stony tissue formation. The outer "fleshy" layer (o.f.), one cell in thickness and just beneath the epidermis, and the inner papery layer (l.p.) may now begin to be differentiated from the middle stony layer (s.t.). × 234. May 9.

Fig. 71b. Details of two cells from Fig. 71a. The normal and active protoplasts are shrunken due to fixation. × 530.

PLATE 5

Fig. 72. Further stage in secondary thickening of stone cells adjacent to inner papery tissue cells (i.p.). p., pit. × 530. May 26.

Fig. 73. Longitudinal section about mid-way the integument of an ovule of the stage of Fig. 67. o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer. × 234. May 26.

Fig. 74. Later stage in secondary thickening of stone cell. Surface view. × 523. June 4.

Fig. 75. Section views of stone cell wall in late stage of thickening. × 1063. p., pit. June 4.

Fig. 76. Stone cells in longitudinal view showing pits and apparently normal and active protoplasts with projections from their periphery into the pit openings. s.w., stone-cell wall. × 490. June 4.

Figs. 77, 78. Transverse and longitudinal views of mature stone cells of the seed coat stained with safranine and Delafield's haematoxylin. The nuclei are still present. × 490. Aug. 5.

Fig. 79. Longitudinal section view of a mature berry-cone to show proportions and relationship of parts. spl., sporophyll; o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer; nuc., nucellus; m-g., megagametophyte; v.-o.s., vascular supply to fruit-scale; v.-c.s., vascular supply to cover-scale; e., embryo; r.c., resin cavity. × 18. Aug. 8.

Fig. 80. Histological details of the portion of Fig. 79 embraced by the two parallel lines and brace near top of figure. r.ca., resin channel. Remainder of abbreviations as in Fig. 79 above. × 56.

PLATE 6

Fig. 81. Group of sporogenous cells (about 8 in all) surrounded by spongy tissue (sp.t.) at base of nucellus. The larger upper cell would probably be the functional megaspore mother-cell. × 530. Mar. 5.

Fig. 82a. Tip of ovule after germination of pollen grain. p.g., pollen grain; mi., micropyle; p.d., pollination-drop; nuc., nucellus. × 163. Mar. 22.

Fig. 82b. Megaspore-mother-cell (m.m.c.) differentiated at base of nucellus in same ovule as in Fig. 82a. × 530.

Figs. 83–87. Triads of megaspores. The megaspores are not generally arranged in straight rows. In Fig. 85, the lowest megaspore is below the level of the other two. (See below.)

Fig. 83. Megaspore triad. × 530. Mar. 22.

Fig. 84. Megaspore triad. × 530. Mar. 26.

Figs. 85, 86. Megaspore triads. × 530. Mar. 24.

Fig. 87. Triad of megaspores. × 1063. Mar. 30.

Fig. 88. Early free-nucleate megagametophyte (n.m-g.) bearing 8 nuclei in all, surrounded by loosely associated spongy tissue (tapetum) and a few nucellar cells. × 234. Apr. 15.

Fig. 89. Later free-nucleate stage of megagametophyte. n.m-g., free-nucleate megagametophyte; t., tapetum; nuc., nucellus. × 234. Apr. 17.

Figs. 90, 91. Prophase and metaphase of division of nuclei in the parietal layer of cytoplasm of the young megagametophyte. × 530. About Apr. 17.

Figs. 92, 93. Comparative sizes of nucellus (nuc.) and free-nucleate megagametophyte (n.m-g.) at the earliest and latest development of the latter. × 16. Fig. 92, Apr. 15; Fig. 93, May 21.

Fig. 94. Proportions and relationship of megagametophytic (m-g.), tapetal (t) and nucellar (nuc.) layers at the time of early wall formation in the megagametophyte. × 234. May 21.

Fig. 95. Division of archegonial initials at summit of megagametophyte (m-g.) to produce the neck initial cell (n.i.) and central cell (c.c.). m.m., megaspore membrane. × 490. May 29.

Fig. 96. Tangential section of young archegonial complex showing an archegonium (ar.) surrounded by jacket layer (tapetum). n., dividing neck cell. × 234. May 26.

Fig. 97. Tier of archegonial neck cells in horizontal plane. × 490. May 29.

PLATE 7

Figs. 98–100. Division of central cell nucleus to cut off ventral canal nucleus (v.c.n.). This division occurs very rapidly, and the ventral canal nucleus disappears quickly after formation. The large central vacuole of the central cell rapidly vanishes while the cytoplasm becomes richer in food material. Meanwhile, the newly formed egg nucleus (eg. n.) enlarges considerably, and the nucleolymph stains light brown with haematoxylin and orange G. × 234. Fig. 98, June 3; Figs. 99, 100, June 2.

Fig. 101. Proportions and relationship of parts of ovule just before divisions of body cell (b.c.) of pollen tube (p.t.) and central cells (c.c.) of archegonia. o.f., outer "fleshy" layer; s.t., stony tissue; i.p., inner papery layer; nuc., nucellus; p.t.n., pollen tube nucleus; t., tapetum; m-g., megagametophyte. × 56. May 29.

Fig. 102. Portion of upper part of an archegonium and adjacent jacket layer (t.) and megagametophyte cells (m-g.) at time of fertilization. s.n., sperm nucleus; s.c., sperm cytoplasm; eg.n., egg nucleus. × 490. June 4.

Fig. 103. Basal part of archegonium soon after fertilization showing sperm nucleus (♂) with its enveloping starch-filled cytoplast in contact with the egg nucleus (♀). Haematoxylin preparation. ar.m., archegonial membrane; p.v., proteid vacuole. × 530. June 4.

Fig. 104. Division of the fusion nucleus (fertilized egg). s.s., starch sheath. × 530. June 6.

Fig. 105a. Archegonial complex showing a zygote with its two basal nuclei invested by the rich supply of starch, resulting from the first division of the fusion nucleus. × 57. June 5.

Fig. 105b. Details of the basal portion of the 2-nucleate zygote of Fig. 105a. × 530.

Figs. 106, 107. Details of the second division of the fusion nucleus and the resulting four daughter nuclei. × 490. Fig. 106, June 5; Fig. 107, June 7.

Figs. 108a, 108b. Archegonial complex (× 58) and details of basal portion of 8-celled proembryo (× 490). Cell wall formation has set in, each cell possessing a nucleus and cytoplast with a rich supply of starch. June 6.

Fig. 109. Eight-celled proembryo, the tier of 4 cells at the morphological base open above. × 490. June 4.

Fig. 110. Divisions (simultaneous) of the tier of 4 cells of the morphological base of the proembryo to produce the open rosette tier above and suspensor tier below. × 490. June 4.

PLATE 8

Fig. 111. Proembryo, with prosuspensors (four in one tier) beginning to elongate. Some of enveloping megagametophyte cells (m-g.) are shown. ps., prosuspensors; s., end cells of proembryo; r.t., rosette tier. × 234. June 7.

Fig. 112. Elongation of the suspensor cells (s.) at the morphological apex of the proembryo has begun. × 234. June 8.

Fig. 113. Later elongation of proembryo. × 110. June 4.

Fig. 114. Morphological apex of proembryo, showing penetration of the megagametophyte tissue by the suspensor units. s., end cells of proembryo. × 234. June 7.

Fig. 115. Later development of proembryo (from paraffin section). The megagametophyte cells adjacent to the tips of the suspensor units (s.) possess a denser cytoplast in which many starch grains are imbedded. m-g.c., sloughed off megagametophyte cells which have become attached to the suspensor cells; p.c., proembryonic cavity within megagametophyte. × 110. June 9.

Figs. 116, 117. Later suspensor units from two different ovules, dissected to show their cellular structure. "1" and "2" of Fig. 116 are obviously the end-cells (s.) of their particular suspensor units. In "3", a prosuspensor cell has just divided. Fig. 117 shows the beginning of proliferation of a secondary embryo (a.e.) from a suspensor cell. All suspensor cells have megagametophyte cells (m-g.c.) adhering tightly to their edges. These are apparently sloughed off mechanically during the elongation of the proembryo. The contents of these cells are used up *in situ*. × 110. June 11.

Fig. 118a. Later proembryo (from paraffin section), showing division of the end cell of a suspensor unit to cut off the embryo initial cell (e.d.) at the tip (leaving the primary suspensor cell immediately above). × 56. June 17.

Fig. 118b. Details of the mitosis of Fig. 118a. × 490.

Fig. 119a. Later proembryo after its suspensor units have cut off their embryo initials. pr.s., primary suspensor; e.i., embryo initial cell. About June 11.

Fig. 119b. Details of tip portion of a suspensor unit of Fig. 119a. (See Fig. 119a.) × 234.

PLATE 9

Figs. 120a–d. Dissected suspensor units at different stages of development. The prosuspensor of a unit, as a rule, seems to be comprised of more cells the nearer it approaches maturity. The proliferation of secondary (prosuspensor) embryos (a.e.) seems to be much less common in the longer proembryos than the shorter ones. pr.s., primary suspensor; e., primary embryo; m-g.c. megagametophytic cells. × 43.

Fig. 121. Daughter cells resulting from mitoses of the embryo initial cell. s.s.i., secondary suspensor initial cell. × 234. About June 16.

Fig. 122a. Later proembryo, entire, showing the characteristically coiled arrangement of the maturing proembryo. pr.s., primary suspensor; e., primary embryo. × 33. About June 18.

Fig. 122b. Details of the terminal embryonic group of cells of the proembryo of Fig. 122a. × 110.

Figs. 123–125. Later primary embryos and their primary suspensors above. That of Fig. 123 is a more typical case. Figs. 123, 125, × 234; Fig. 124, × 110. June 18-24.

Fig. 126a. Later development of a whole proembryo. Embryonal tubes (cells) are growing back from the morphological basal part of primary embryo to produce the secondary suspensor. × 33. About June 25.

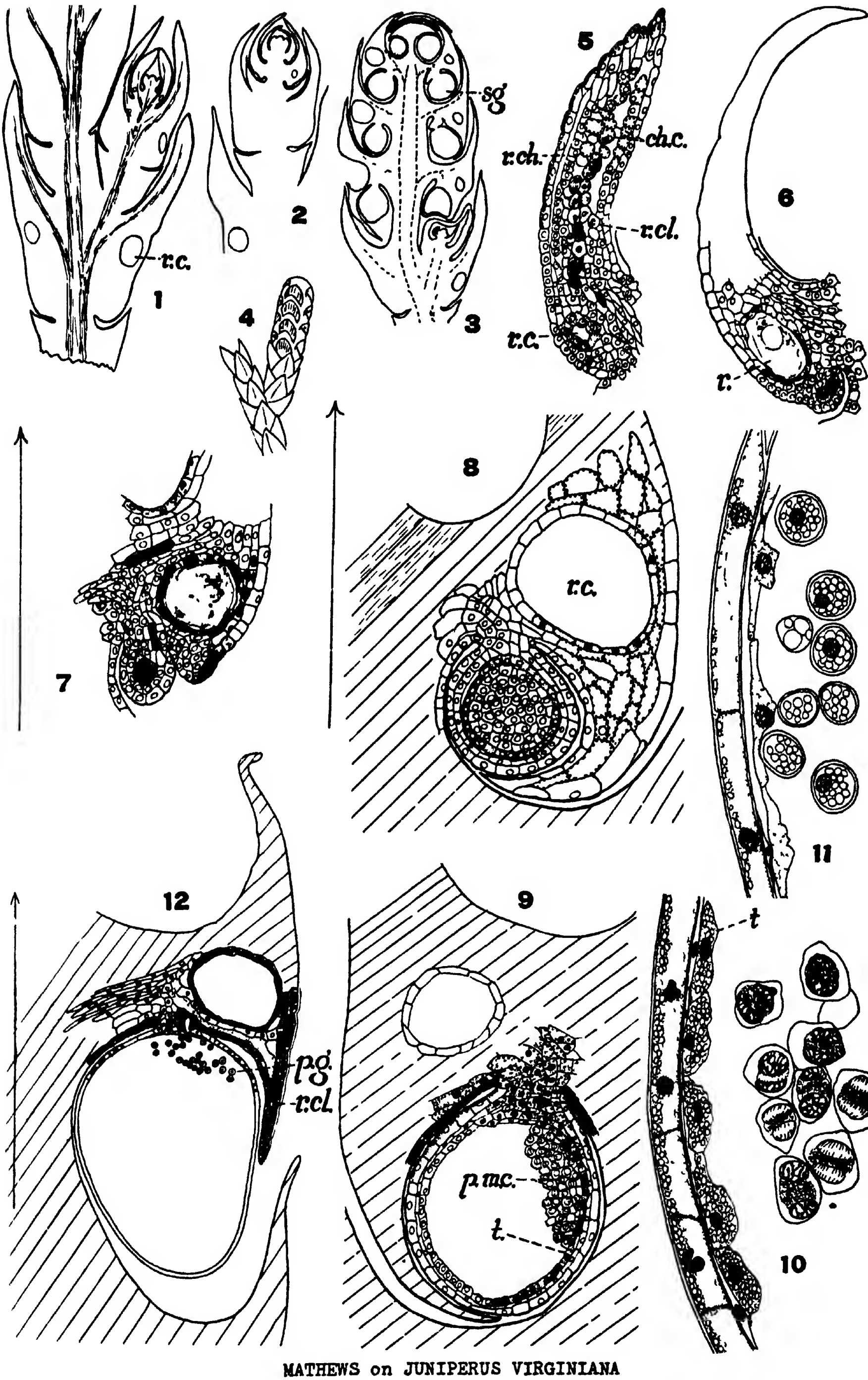
Fig. 126b. Details of the terminal primary embryo (e.) of Fig. 126a. pr.s., primary suspensor; e.t., embryonal tubes (cells). × 110.

Fig. 127. Later primary embryo. The primary suspensor seems to be dislodged from its original attachment and pushed backward by the developing secondary suspensor cells. × 110. About June 25.

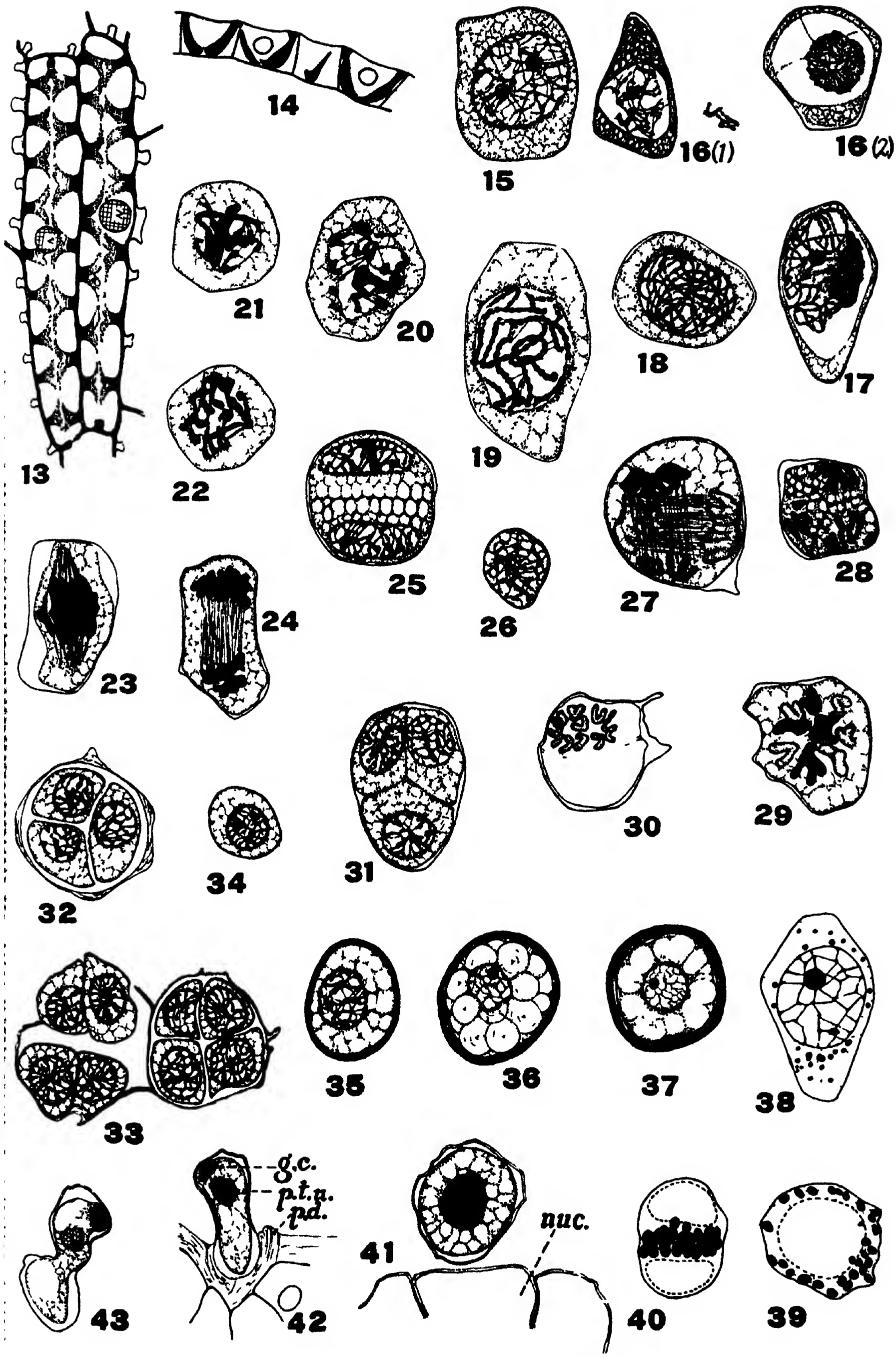
Fig. 128. Later proembryo, showing further development of the functional primary embryo (e.) and the secondary suspensor (s.ss.) above. pr.s., primary suspensor; ps., prosuspensor. × 33. About July 20.

Fig. 129. Early appearance of the plumule (plu.) and the two cotyledons (cot.). Many nuclei are observable in the old prosuspensor units above but are not shown here. × 33. About July 24.

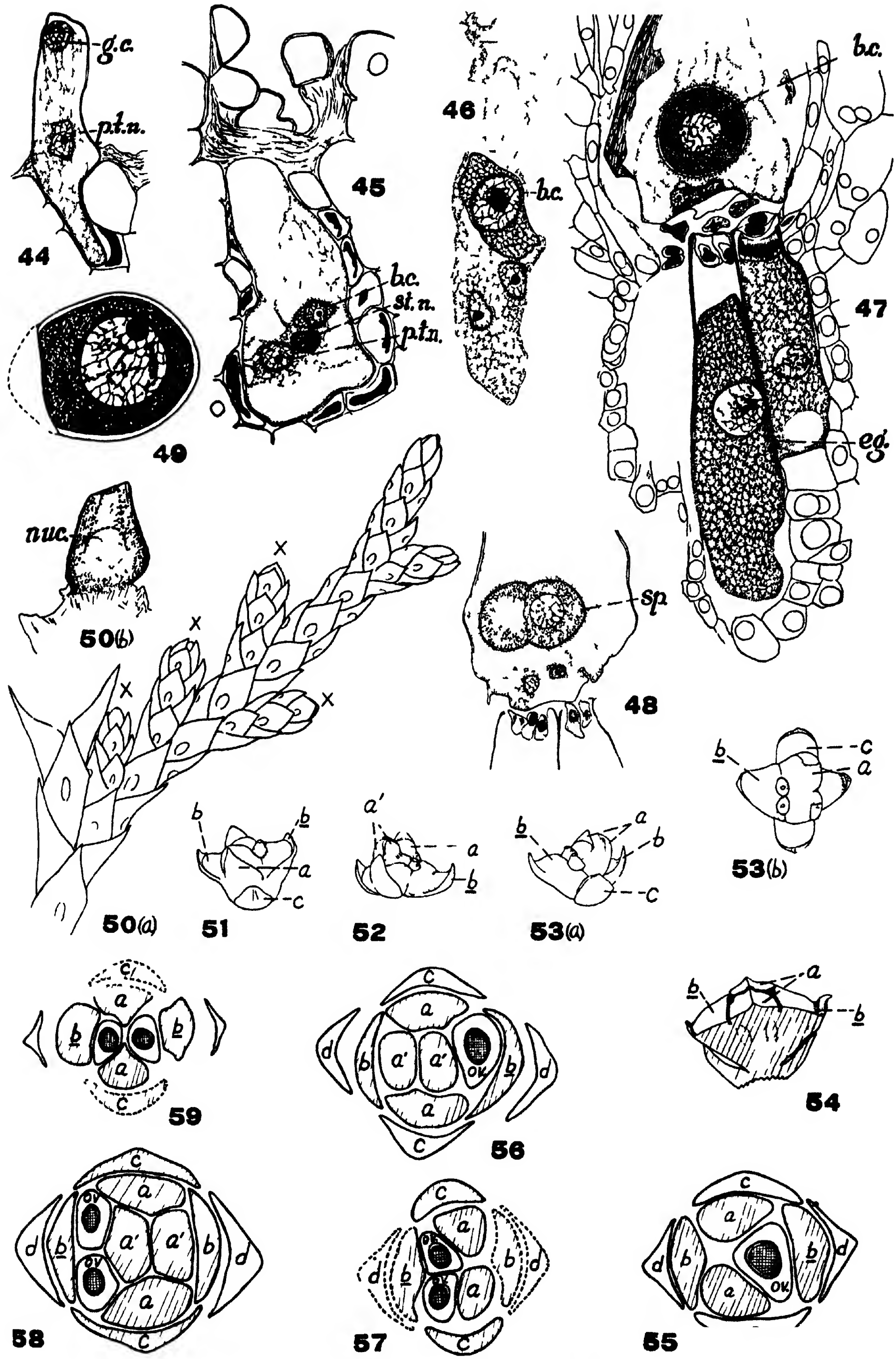
Fig. 130. Mature primary embryo. The parallel lines indicate the arrangement of the peripheral cell layers. plu., plumule; cot., cotyledon. × 24. July 28.



MATHEWS on JUNIPERUS VIRGINIANA

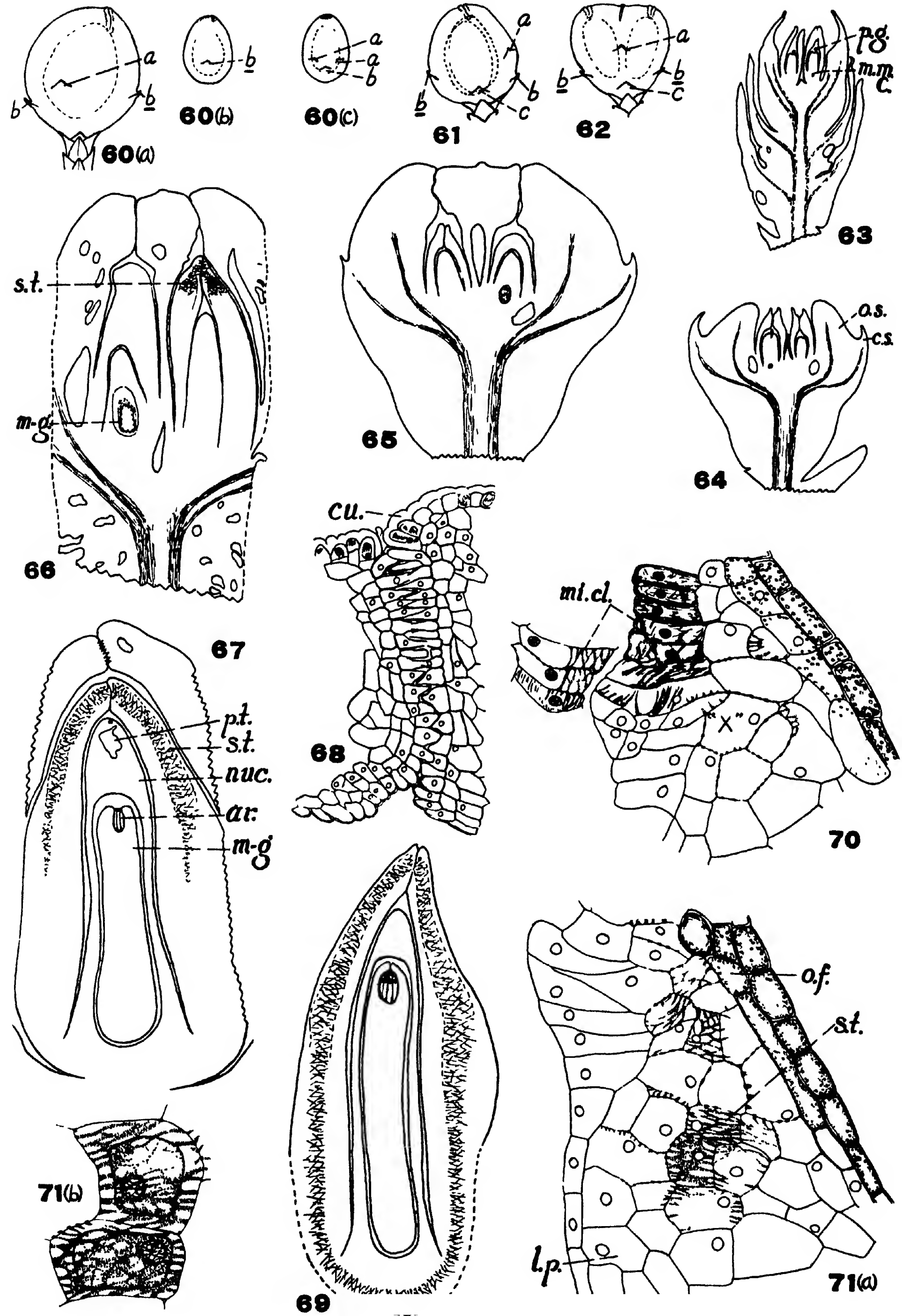


MATHEWS on JUNIPERUS VIRGINIANA



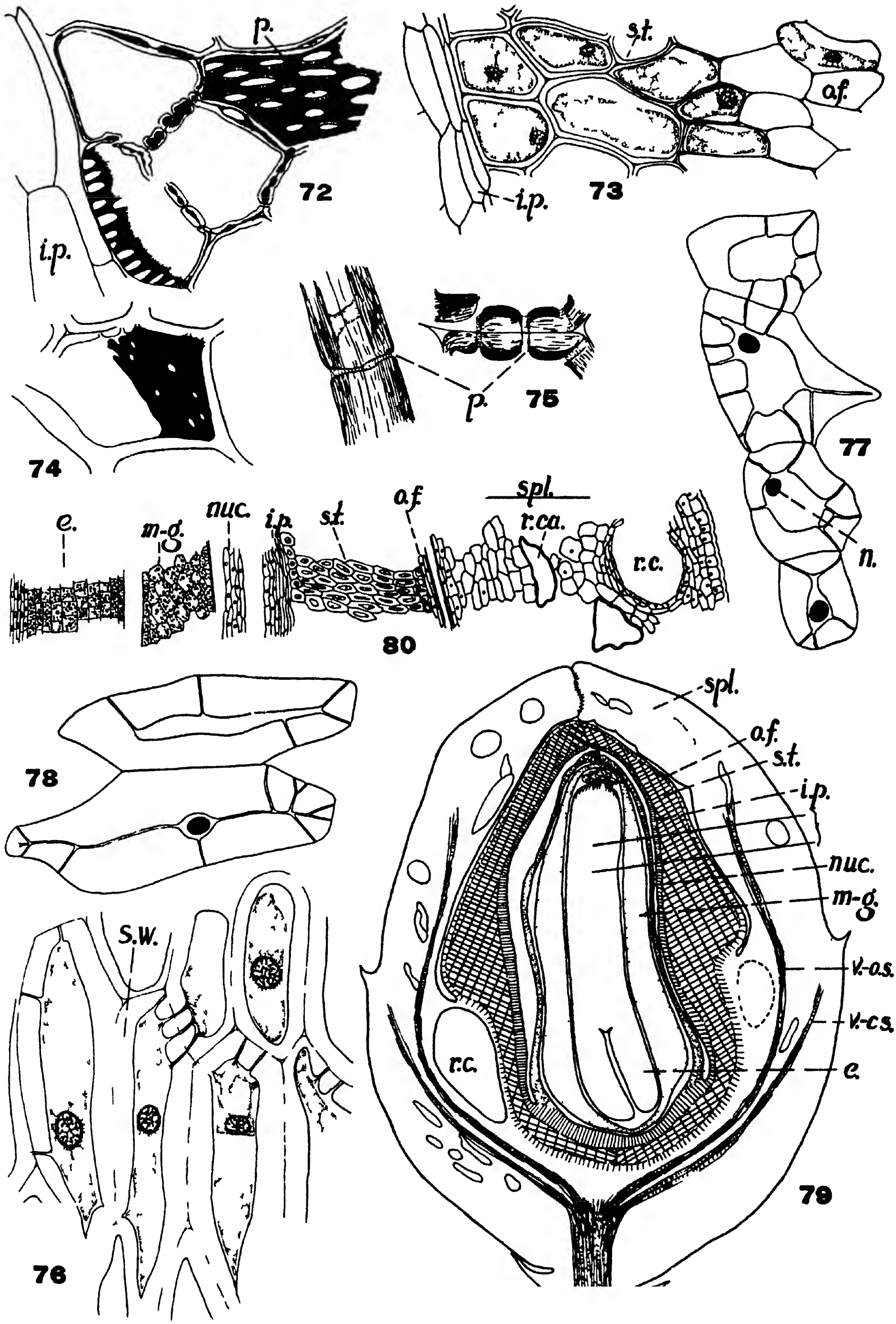
MATHEWS on JUNIPERUS VIRGINIANA

PLATE 4



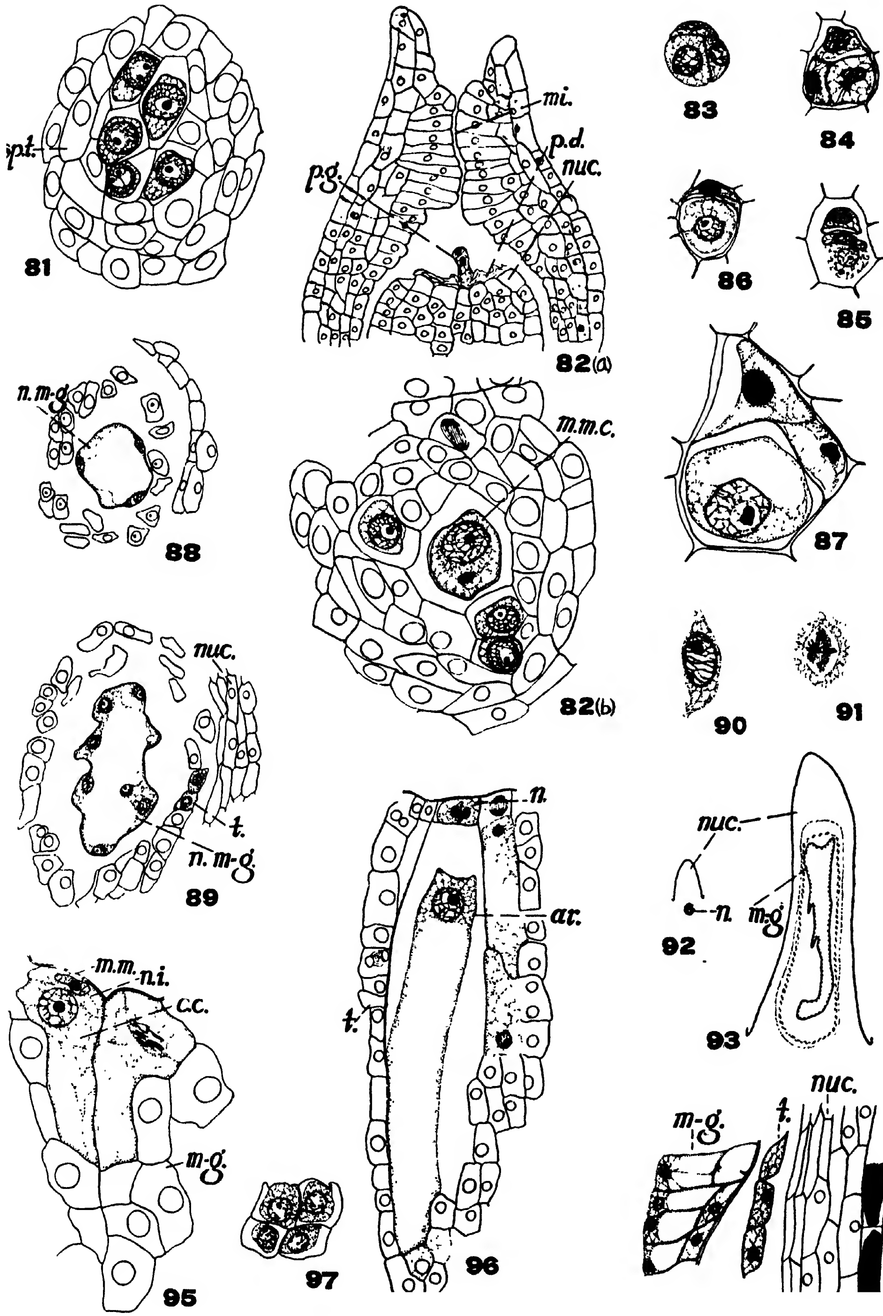
MATHEWS on JUNIPERUS VIRGINIANA

PLATE 5

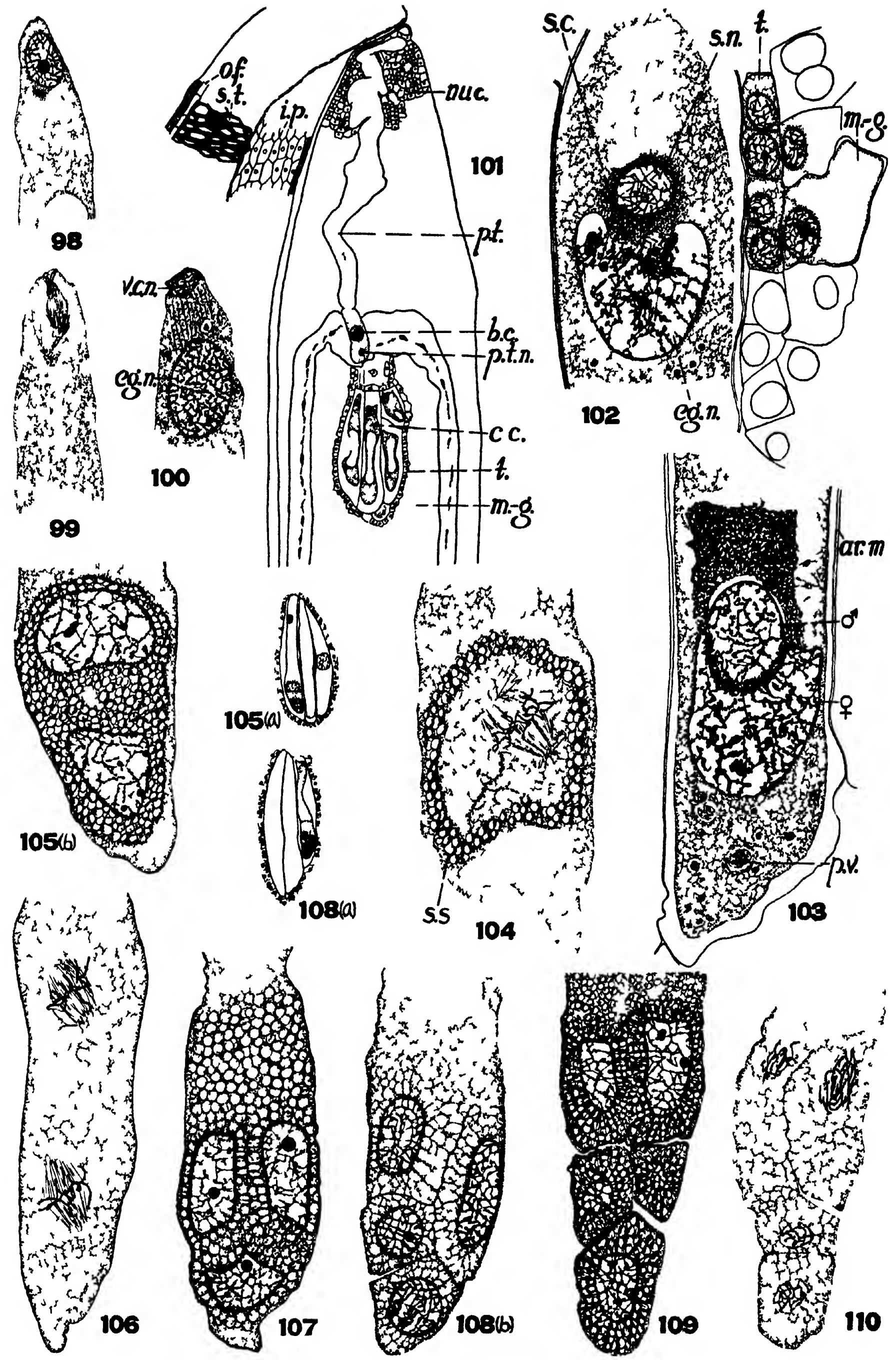


MATHEWS on JUNIPERUS VIRGINIANA

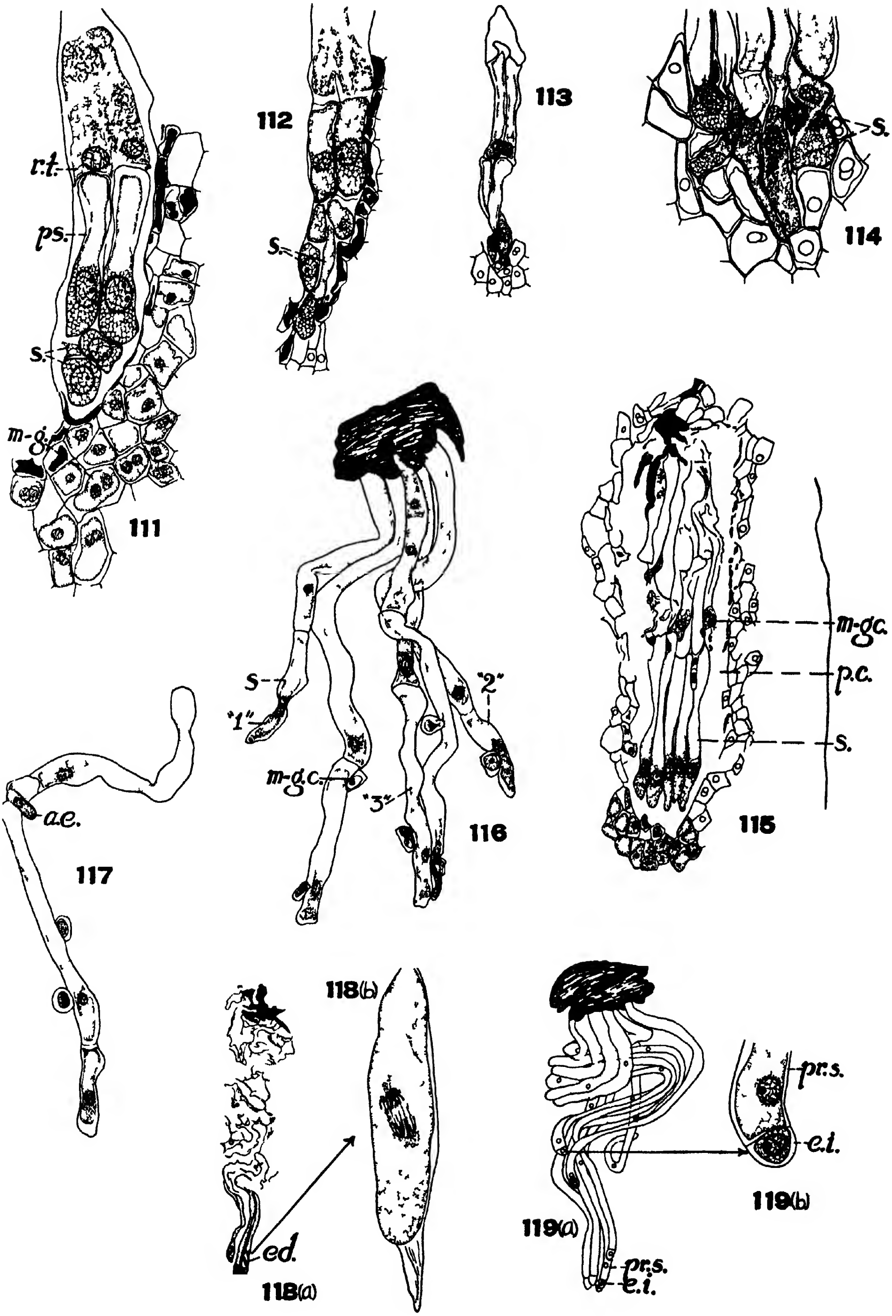
PLATE 6



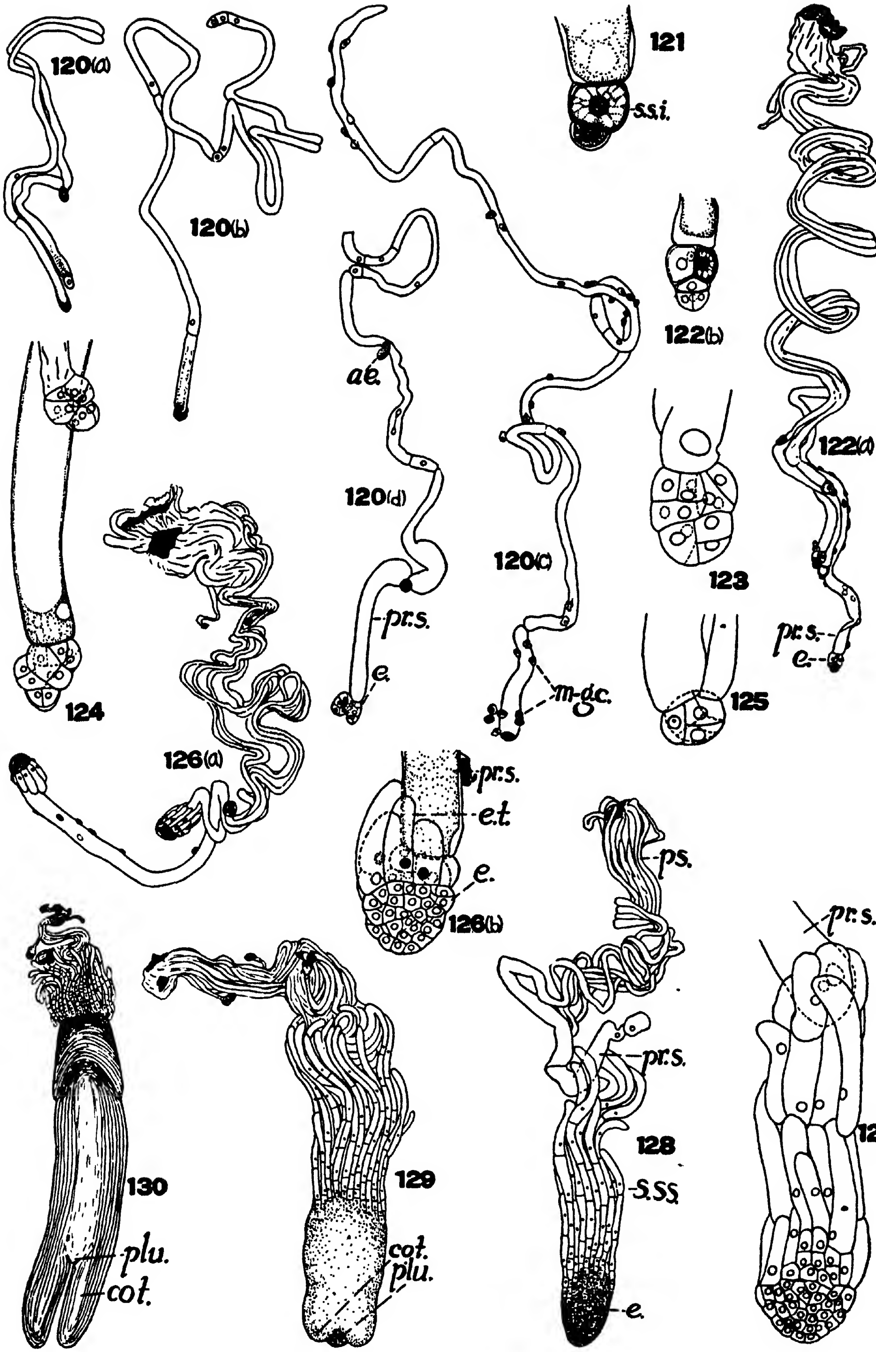
MATHEWS on JUNIPERUS VIRGINIANA



MATHEWS on JUNIPERUS VIRGINIANA



MATHEWS on JUNIPERUS VIRGINIANA



MATHEWS on JUNIPERUS VIRGINIANA

SEED DEVELOPMENT OF LOBELIA AMOENA*

By W. C. Hewitt

Plates 10–13

Historical Introduction

In 1878 Warming (42) published two figures of the young ovule of *Lobelia Dortmanna:* one with the megaspore mother cell about half grown and lying in almost a straight line with the funiculus, and with the primordium of the integument showing; the other of a nearly mature megaspore mother cell lying parallel with the funiculus, and the integument developed well beyond the outer end of the single-layered nucellus.

In the same year Vesque (39) gave the number of megaspores in *Lobelia laxiflora* as usually four but sometimes three. He also showed a mature egg apparatus of *Siphocampylus Manettiaeflorus* consisting of two elongated synergids and an egg cell, with a fusion nucleus just below, and an embryo sac 'tapetum' well developed.

In 1880 Ward (41) showed that the number of megaspores in *Lobelia syphilitica* is three, or four with the lower two lying side by side. Most of his paper is concerned with a 'cell-plate' which he showed passing transversely through the central vacuole of the two-nucleate embryo sac. None of his other figures showed such a plate. He showed figures of the mature sac both before and after the polar nuclei fuse to form the 'nucleus of the embryo sac'; and in his most mature figure shows the three antipodals disintegrating, but does not mention this in the text.

In 1881 Jönsson (17) described the development of the embryo sac in *Lobelia Dortmanna.* And for *L. Aubrietiae* he described the development of the megaspores from the megaspore mother cell, and described the mature sac. He also showed that the embryo sac develops from the lowest of the megaspores in *L. erinus.*

In 1882 Guignard (15) showed three megaspores for *L. erinus,* but said it is possible that a fourth develops; in any case it is the chalazal spore which develops into the embryo sac. He mentioned the early disap-

*A thesis submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Master of Arts in the Department of Botany.

pearance of the nucellus, and said that the upper end of the sac with the egg apparatus steals into the micropyle.

In 1896 Schlotterbeck (30) considered the transformed antipodals as playing a part in the formation of the chalazal haustorium of *Lobelia inflata.*

In 1901 Billings (5) showed a figure of the mature embryo sac of *Lobelia excelsa* with only the remnants of the antipodals. He described the synergids as being long with no nuclei (but had only one mature sac to study). He showed a well developed 'tapetum' extending from the lower end of the nutritive tissue to the upper tip of the embryo sac and showed a fibro-vascular bundle extending up into the funiculus. He described the early development of the endosperm as dividing first transversely, then by a longitudinal division in each of the resulting cells, the upper cells moving around the elongating egg cell which extends into the micropyle with them. He showed that the micropylar and chalazal haustoria each develop from two cells of the endosperm but did not show which cells develop into the large central mass of endosperm. For *L. Cliffordiana* he described the micropylar haustorium as developing from only the nuclei-bearing projections of the two upper cells and the chalazal haustorium as developing from a similar projection from a single lower endosperm cell.

In 1912 Armand (1) gave the haploid number of chromosomes as eight for *Lobelia erinus, L. urens,* and *L. Dortmanna.* He described the exine of the pollen for these species as having three pores of germination and germinating very easily in distilled water. He also described the grain as containing a granular protoplasm with a spherical vegetative nucleus rich in chromatin and an elongated generative nucleus surrounded by clearer protoplasm. He said that when the tube is 20 times as long as the diameter of the spore the vegetative nucleus disappears and the generative nucleus divides. These species show a single integument, very small nucellus, and a single hypodermal archesporial cell which gives rise to four megaspores, the lowest of which (sometimes the third) develops into the embryo sac. He described the embryo sac before the fusion of the polar nuclei and said that fertilization occurs in the usual way: the tip of the pollen tube touches the superior part of the embryo sac; the membranes in contact disappear; the synergids swerve; and the slightly elongated male gametes appear to enter them. He observed double fertilization, the polars not fusing until fertilization occurs. And according to him in these species, the fertilized egg elongates and divides transversely, the superior cell dividing transversely

to form a 4–8-celled suspensor, and the inferior cell dividing by three perpendicular walls to form eight cells which then divide by tangential walls to form a 16-celled embryo. He was not able to follow the development further but said that the mature embryo is small and is composed of undifferentiated cells.

In 1932 Rosen (28) described the development of the micropylar haustorium of *Lobelia Dortmanna* as developing from the upper daughter cells of the first two micropylar endosperm cells, and described the development of the endosperm as being cellular from the very beginning.

In 1935 Kausik (18), for *Lobelia trigona*, briefly described the development of the ovule, megaspores and embryo sac. The single layer of nucellus breaks down at the four-nucleate stage of the sac, the inner layer of the integument developing into a 'tapetum' which encloses the nutritive tissue at the chalazal end of the sac. The fusion nucleus is formed before fertilization. He described the divisions in the endosperm as being first transverse, followed by longitudinal divisions in each cell, the latter divisions being perpendicular to each other. The two lower cells develop directly into the chalazal haustorium; the two upper cells divide transversely, the upper daughter cells developing into the micropylar haustorium and the two lower daughter cells dividing to form the massive endosperm. He described the divisions of the embryo as being first transverse followed by three transverse divisions in each cell, making a four-celled embryo and a four-celled suspensor. He showed that the terminal cell of the four-celled embryo undergoes the next division by a longitudinal wall, followed by the division of the next cell also in the same plane. He said that the first three transverse divisions in the embryo cell can still be seen in late stages of the embryo, but has very few figures to illustrate this. He described in some detail the outer cells of the integument which become elongated and develop elaborate thickenings along their inner and lateral walls. In this paper he paid special attention to the nutrition of the embryo sac.

As far as seed development is concerned, the writer has found no mention of *Lobelia amoena* in the literature, and no work on the Lobeliaceae published in this country. In the literature cited above (except 1, 18) a brief account of one or more species of the Lobeliaceae was included in a general discussion of some particular phase of seed development. No full account of the seed development in any species of the family has been found.

The present work was done under the direction of Drs. W. C. Coker and H. R. Totten, and for this direction the writer is grateful. The

writer also wishes to express appreciation for helpful suggestions from Drs. J. N. Couch, Velma Matthews, and Vera Millsaps; and to Misses Alma Holland, Evelyn Mullen, and Constance Sherman for help in locating references in the literature and translating the articles.

MATERIALS AND METHODS

Material was collected around Chapel Hill, N. C., throughout September and October 1934; from Elizabeth City, N. C., in August 1935; from Chapel Hill during July and August 1937; and around Hartsville, S. C., during September, October, and November 1938. Material for sectioning was killed in weak chromo-acetic solution, formalin acetic alcohol, and a combination of Carnoy's and Nawaschin's fixing fluids. All of the best material was killed and fixed in the latter combination. The material was allowed to stand in Carnoy's for about ten minutes then changed to Nawaschin's fluid which was allowed to react over night.

The tissues were dehydrated through the ethyl alcohol series, and through the diethyl ether series. In nearly all cases the latter gave better results, but for the young embryo sac the alcohol series gave better results.

Haidenhain's iron-alum haematoxylin, Flemming's triple, and safranin-gentian violet stains were used. The safranin-gentian violet stain gave the best results.

Microspore mother cells and stages showing the divisions of these cells to form the microspores were killed and stained by Billing's iron acetic carmine technique. This gave good results on these stages.

The pollen grains and pollen tubes were stained by Feulgen's technique as described later in this paper.

Most of the drawings in this paper were made from material which was killed in a combination of Carnoy's and Nawaschin's fluids, dehydrated through the ether series, and stained with safranin-gentian violet. The drawings were made at table level with the aid of a Spencer camera lucida and reduced.

STRUCTURE OF THE FLOWER

The flower is considered epigynous although the top part of the ovary is not covered by any of the other floral parts. The stamens, petals, and sepals all arise from a common projection about midway between the top and bottom of the ovary. The anthers are joined together side by side forming a tube, but the filaments are separate. The petals

are fused together forming a long tube which is two-lipped. The sepals are fused together at the very bottom with the upper parts projecting as five long narrow lobes. The pistil is compound, being composed of two locules, a long style, and a two-lobed stigma. Before the lobes of the stigma open to expose the stigmatic surface, the outside of the young stigma is covered with long bristles which brush the pollen out of the pollen sacs as the stigma grows up through the anther tube. The placenta is axial with each locule containing a large lobe which is covered with numerous anatropous ovules.

MICROSPORANGIUM AND DEVELOPMENT OF MALE GAMETOPHYTE

The general appearance of the anther in cross section is typical. There are four microsporangia, each containing a mass of microspore mother cells surrounded by four rows of wall cells (Fig. 1).

The microspore mother cells are cuboidal, containing a distinct nucleus and small vacuoles. Later the contents of these cells become more homogeneous; the vacuoles disappear; and the nucleus gets larger. The two divisions of the mother cell are in rapid succession, the second division occurring before the spindle of the first is gone (Figs. 2–4); and both nuclear divisions occurring before wall formation begins (Fig. 5). When the young spores are first formed most of the cell is filled with a large vacuole, the spore at this stage resembling a typical fat cell with only a very narrow band of cytoplasm around the outside of the vacuole (Fig. 6). On one side of the vacuole the cytoplasm is a little broader, and it is here that the nucleus is located. Similar vacuolated young microspores have been described by Locke (20) for *Asimina triloba* and by Madge (21) for *Viola odorata*. As development continues the entire cell becomes filled with rather granular cytoplasm with tiny vacuoles scattered throughout (Fig. 7). The microspore then divides in the usual way to form two cells of unequal size (Fig. 8). However, this division often occurs while the microspore nucleus is on one side of the cell with only a few bands of cytoplasm crossing the large vacuole. The smaller cell, the generative cell, is located on one side of the spore when it first appears and is separated from the tube cell by a distinct membrane. Soon the generative cell moves away from the outside of the spore and becomes entirely surrounded by the contents of the larger tube cell (Fig. 9). The membrane of the generative cell still surrounds its cytoplasm after moving into the vegetative cell, but this membrane disappears very early. The nucleus of the generative cell has the appearance of a resting nucleus

for only a short time, soon becoming oblong in shape and smaller in size, with the nucleolus disappearing and large chromatin granules appearing which are scattered throughout the nucleus. When first formed the cytoplasm of the generative cell is not very dense, having numerous small vacuoles scattered throughout its contents. After moving into the center of the pollen grain this cytoplasm changes into a dense spindle-shaped mass surrounding the nucleus and projecting at each end of the nucleus. This leaves a space surrounding the dense cytoplasm that does not contain any granules and does not stain (Fig. 9). When the cell membrane of the generative cell disappears, the granular cytoplasm of the vegetative cell moves in until it touches the dense cytoplasm of the generative cell; but the cytoplasm of the generative cell is denser and still can be recognized as a spindle-shaped mass until the formation of the pollen tube begins (Fig. 10).

When this change is taking place in the generative cell, the nucleus of the vegetative cell is disappearing, the nuclear membrane disappearing first. Later the entire nucleus including the nucleolus disintegrates, leaving for a time small fragments of deeply staining material scattered in the cytoplasm of the cell. The tube nucleus usually entirely disappears before the pollen tube begins to develop, but in a few cases a nuclear mass about the size of the nucleolus of the tube nucleus was seen in the pollen grain with the generative nucleus as the tube began to develop. In unusual cases one or more fragments from the tube nucleus could be distinguished in the tube; however at this time they stained very little darker than the cytoplasm of the tube.

As early as 1879 Elfving (13) thought that the tube nucleus degenerated before the germination of the pollen grain in *Hypericum calycinum*. The degeneration of the tube nucleus in the pollen grain has been reported by Shattuck (33) 1905, for *Ulmus americana*; by Poddubnaja-Arnoldi (26) 1927, for *Echinops sphaerocephalus*, *Carduus crispus*, and *Centaurea scabiosa*, and (27) 1933, for *Senecio platanifolius*; by Sax and Edmonds (29) 1933, for *Tradescantia*; and by Cooper (11) 1938, for *Pisum sativum*. The tube nucleus has been reported to show signs of disintegration either in the mature pollen grain or early in the tube by Osterwalder (25) 1898, for *Aconitum napellus*; by Barnes (3) 1885, for *Campanula americana*; by Armand (1) 1912, for three species of *Lobelia*; by Wylie (44) 1923, for *Vallisneria spiralis*; by Finn (14) 1928, for *Vinca minor*; by Madge (21) 1929, for *Viola odorata*; by Cooper (9) 1935, for *Chenopodium hybridum*, *C. album*, *Atriplex patula* var. *hastata*, and *Salsola kali*. There are a large number of cases which

show some evidence of a degenerating tube nucleus, and the writer is of the opinion that this degeneration is more common than is often believed. Wulff and Maheshwari (43) express some doubt as to whether in reported cases the tube nucleus does actually disappear or whether its apparent absence may be due to failure to stain. We have no doubt that in the case of our plant, the disappearance of this nucleus is actual and not merely apparent.

However, in the numerous botanical textbooks that have been examined during this study, including the latest ones on cytology, no mention is made of the not-so-infrequent early disappearance of the pollen tube nucleus in angiosperms, and it is often stated that this nucleus is supposed to direct the growth of the pollen tube. The full development of the pollen tube in the complete absence of the pollen tube nucleus brings up the interesting theoretical question of the ability of generative nuclei to function vegetatively as well. This is of course assuming that the influence of a nucleus is needed for vegetative growth.

To sum up, at the time that the pollen grain of *L. amoena* is shed it usually contains a generative nucleus surrounded by a definite mass of cytoplasm and only fragments of the tube nucleus, if there is any of it remaining at all. In the pollen tube it is only in exceptional cases that even obscure fragments that might have come from the degenerating nucleus, can be found. Its wall consists of two layers, a heavy exine and a very thin intine. Three elongated pores have developed in the exine, where the intine projects outward beyond the general surface of the spore (Fig. 10).

The pollen grains germinate very easily in a 5% sugar solution or in distilled water, but in distilled water the tubes do not grow long before they burst. In either case the germination begins almost at once. As soon as the tube projects through the exine, the cytoplasm on the inside can be seen in constant motion, moving from the spore into one side of the tube, up the other side of the tube and back into the spore. By the time that the tube grows in length equal to about twice the diameter of the spore, the contents within the spore itself become so thin that their movements are visible. A similar streaming of the cytoplasm in the young tube was described by Barnes (3) for *Campanula americana*. The contents of the spore become very vacuolated, the vacuoles soon extending down into the tube.

For detail study of the pollen tubes, the grains were germinated and stained according to the directions given by Lee (19). A thin coat of

melted agar-sugar solution (1.5 grams agar, 5 grams cane sugar, 100 cc. distilled water) was smeared on the slide, and the pollen grains were sifted into the solution before it hardened. They were germinated in a moist chamber from 2–15 hours. These tubes were then stained by the Feulgen's technique.

The generative nucleus does not pass into the tube until the tube has grown longer than the diameter of the grain. At the time this nucleus enters the tube, there is usually no distinct mass of cytoplasm surrounding it (Fig. 11). The generative nucleus divides to form the two oblong or irregular-shaped male nuclei in the tube after the tube is very long (Fig. 13), this division occurring when the generative nucleus is about the same distance from the tip of the tube as is shown in figure 12.

DEVELOPMENT OF THE OVULES

The ovules begin as little protuberances on the surface of the placenta, and up until the time of bending the entire ovule is an undifferentiated mass. Very early, however, a single hypodermal cell enlarges and reacts differently to the stains. This cell, identified as the archesporial cell, becomes very conspicuous and functions directly as the megaspore mother cell, which is the usual procedure for the Sympetalae (12). When the mother cell is first recognized, the integument has already begun developing on the outer side of the curving ovule (Fig. 14). The mother cell is covered by only one layer of nucellus. This cell elongates to two or three times it original size before its first division (Fig. 15).

MEGASPORES

The megaspore mother cell divides transversely to form a dyad (Fig. 16); then each of these cells divides in the same plane to form a linear tetrad of spores (Fig. 17). It was not determined which of the divisions is the reduction, but the megaspores themselves contain the haploid number of chromosomes, seven in this species. In the usual way the three spores nearest the micropyle soon disappear while the lower one grows larger (Figs. 18, 20). Although this is the usual procedure, a few cases have been found in which only three megaspores are formed, the upper cell of the dyad not dividing to form two spores but containing two nuclei (Fig. 19). In these cases the spore which elongates to form the embryo sac is the one farthest from the micropyle. In one case the second and fourth spores, counting from the micropyle, were both dividing to form an embryo sac (Fig. 21). The second spore

was ahead, having already formed a two-nucleate sac with the nuclei in opposite ends of the sac while the nucleus of the fourth spore was still in the telophase stage of mitosis. The collapsed first and third cells could be easily seen. No other stages in such a development were found.

THE EMBRYO SAC

The development of the embryo sac follows the monosporic 8-nucleate normal-type described by Maheshwari (22) and other recent writers. The chalazal megaspore, containing a vacuole in each end, enlarges until it is as large as the four original spores before it begins to divide (Fig. 20). As soon as the first division is complete one nucleus moves to each end of the sac, and the vacuoles change from the ends to a single large vacuole in the center (Figs. 22, 23). Figures 24 and 25 show four- and eight-nucleate sacs. Soon strands of cytoplasm cross the large central vacuole of the sac, and three cells are cut off at each end, leaving the two polar nuclei in the large central part. The three antipodals look almost like an inverted egg apparatus when they are fully developed, but they disappear very early. Figure 26 shows the egg apparatus (only one synergid in this section) at the upper end of the sac, and the three antipodals at the lower end. The two polar nuclei move towards each other and fuse in the upper end of the sac. In this fusion the nucleoli remain distinct for some time after the nucleoplasm mixes (Figs. 27, 28). When the egg apparatus is mature the antipodals are disappearing (Fig. 29). At this time each of the synergids is much longer than wide and contains a large vacuole which fills the lower half of the cell, the nucleus lying in the upper half close to the vacuole. The micropylar ends of the two synergids break through the embryo sac and push up into the micropyle. The egg cell is much shorter than the synergids; and its lower half, containing the densest cytoplasm, extends below the synergids. Figure 30 shows a cross section of the synergids above their nuclei; here neither the egg cell nor the sac is present.

NUCELLUS

The part of the nucellus which covers the sporogenous tissue is never more than one layer thick. The cells of this layer are cuboidal when first observed and in general give the appearance of being in a healthy condition (Fig. 14). Soon after the megaspore mother cell begins to elongate, the cells of the nucellus stop multiplying, become

almost void of cytoplasm, and give the appearance of being stretched (Figs. 15–20). The four megaspores are not much larger than the large mother cell which gave rise to them, and the functional megaspore does not exceed the size of the four original megaspores until its first division. The two-nucleate sac increases in size to about one and a half times the size of the four original spores. It is during this increase in size that the sac splits the nucellus at its upper end, and the upper end of the two-nucleate sac moves out into the space surrounded by the integument (Fig. 22). Bushnell (6) has described a similar change in the appearance of the embryo sac of *Monarda fistulosa* following the first division of the sac nucleus, and she has shown that the two-nucleate sac breaks through the nucellus. The layer of nucellus which originally surrounded the upper end of the mother cell and the four spores rapidly disappears, and by the time that the sac is mature it is entirely gone. The nucellus (nutritive tissue) at the lower end of the embryo sac is present until the endosperm is formed. The endosperm forces its way down into this part of the ovule and develops into a large haustorium which absorbs all of the nucellus before the zygote divides.

INTEGUMENT

There is a single integument, the growing point of which may be seen at the time when the ovule first begins to bend. The integument is first seen on the side of the ovule farthest from the funiculus (Fig. 14). The cells of the very young integument are large; as they increase in number, they get smaller (Figs. 14, 15, 17). The integument grows rapidly. By the time the mother cell is mature, the integument has grown out beyond the nucellus at the upper end (Fig. 15); and by the time the functional megaspore is enlarging the integument has grown out beyond the nucellus so far that the upper end of the nucellus seems to lie about midway between the upper and lower ends of the ovule. The integument continues its development until the endosperm is formed.

'TAPETUM'

The inner layer of cells of the integument becomes distinguished as a 'tapetum' as early as the megaspore stage, even before the three micropylar megaspores begin to disappear (Fig. 17). At the time that the functional megaspore is ready to divide, the 'tapetum' is well developed from the place where the integument joins the nucellus at the lower end to a few cells beyond the upper end of the nucellus (Fig. 19).

As soon as the nucellus disappears, the 'tapetum' lies next to the embryo sac. The 'tapetum' reaches its maximum development about the time of fertilization. At this stage the cells of the 'tapetum' are long and narrow with their longitudinal axis perpendicular to the longitudinal axis of the sac. At this stage the tapetal cells contain large nuclei which do not stain as clearly as the other nuclei of the integument, but the rest of the tapetal cell stains much darker than the surrounding cells of the integument. The cells of the 'tapetum' increase in number from the time that they first appear until the time of fertilization, additional cells being cut off in the 'tapetum' and new cells being added on at the micropylar end. But this increase in length of the 'tapetum' does not keep pace with the rapid increase in length of the developing embryo sac; therefore the upper end of the mature sac projects out beyond the 'tapetum.' The part of the embryo sac which increases in width is the part which is not surrounded by 'tapetum' (Fig. 26). From the appearance of the cells of the integument surrounding the upper end of the sac above the 'tapetum,' the sac digests its way up into the micropyle. As soon as the endosperm begins forming, the entire 'tapetum' begins collapsing (Figs. 38–40).

FERTILIZATION

The pollen tube grows down through the micropyle, destroys one of the synergids, and discharges its contents into the embryo sac. One of the male nuclei was seen in different stages of fusion with the fusion nucleus (Figs. 33, 34), and the other male nucleus was seen fusing with the egg nucleus (Fig. 32). Usually the two polar nuclei fuse before fertilization, but often this fusion is delayed until fertilization; then one male nucleus and the two polars fuse at the same time (Fig. 31). In both the endosperm nucleus and the zygote nucleus, the nucleoli remain distinct for some time after the fusion of the nucleoplasm (Figs. 33–36). Johansen (16) has described a similar fusion of the gametes of *Clarkia elegans*. The chromosome number in the cells of the integument is 14, and the number in the cells of the endosperm is around 20. These figures are approximately twice and three times the haploid number, seven, counted in the embryo sac.

ENDOSPERM

The endosperm nucleus divides before the sperm and egg nuclei completely fuse (Fig. 35). The first division of the endosperm is perpendicular to the longitudinal axis of the sac. A cell wall is formed

after the first division of the nucleus, and there are no free nuclear divisions in the endosperm. The next division is longitudinal in each of these cells with the cell walls in the two cells almost perpendicular to each other. Figures 36 and 37 were made from ovules cut obliquely so that all four of the cells were showing. In many cases only one cell of either the lower two or upper two shows in a single section (Figs. 38–40). Now each of these four cells divides transversely forming four tiers of two cells each (Fig. 37). The two cells of the upper tier never divide again but move up around and beyond the zygote and force their way up into the micropyle, destroying what is left of the egg apparatus except the egg (zygote), and forming a large micropylar haustorium (Figs. 38–40). The two cells of the lowest tier do not divide again either, but move down into the chalazal end of the ovule, destroying the nutritive tissue, and developing into a large chalazal haustorium (Figs. 38-40). Cross sections of mature haustoria, both micropylar (Fig. 42) and chalazal (Fig. 41), show that they are composed of these two original cells. The cells of the two middle tiers multiply rapidly to form the massive endosperm of the seed (Figs. 38–40). About twenty cells are formed in the endosperm and the two endosperm haustoria are rather well developed before the first division of the zygote (Fig. 38).

The origin of the chalazal haustorium and the central mass of endosperm as described above is not in agreement with the account given for *Lobelia trigona* by Kausik (18), who is the only writer having discussed the origin of these structures for the Lobeliaceae. The origin of the micropylar haustorium is, however, in agreement with the account given by Rosen (28) and Kausik (18).

EMBRYO

The first division of the zygote is transverse, cutting off what later proves to be a primary embryonal cell *e* and a primary suspensor cell *s* (Fig. 44). In all cases the suspensor cell is the first of these to divide; this is also a transverse division (Fig. 45) cutting off a middle cell *m* and a basal cell *l*. Often these two cells will divide again by transverse walls before the embryo cell divides; but the most common procedure is for the embryo cell to divide first by a transverse wall (Fig. 46) cutting off an apical cell *a*, which does not divide again until longitudinal divisions begin, and a second embryonal cell *f*. The cells *m*, *l* divide by transverse walls forming a four-celled suspensor *n*, *o*, *p*, *h* (Figs. 47–49). Each of these suspensor cells may divide again, the divisions

in the distal end being either transverse or longitudinal, producing a suspensor *s* about 8–12 cells in length (Figs. 50–55). The second cell *f* of the embryo divides by a transverse wall to form cells *b*, *g* (Figs. 48, 49). The cells *a*, *b* next divide by longitudinal walls, and the cell *g* divides by a transverse wall. Either of these cells may be the first to divide, but the product is always the same: two cells side by side in both *a*, *b* and two superimposed cells *c*, *d* formed from *g* (Figs. 49, 50). The first vertical divisions in *a*, *b* are perpendicular to each other (Fig. 50). The embryo now consists of four tiers of cells *a*, *b*, *c*, *d*, and these four tiers can be traced throughout the development to the mature embryo (Figs. 50–56). The next division in both *a*, *b* is vertical and perpendicular to the first division in that tier (Fig. 51). Cell *c* divides by a vertical wall (Fig. 51). This produces two tiers of four cells each at the distal end followed by a tier of two cells *c* and a tier of one cell *d*.

In the distal tier *a*, anticlinal divisions occur first (Fig. 52) followed by periclinal divisions (Fig. 53) which cut off the dermatogen in this region of the embryo. The first anticlinal divisions in this tier can be traced in the development to the mature embryo. These anticlinal divisions separate the future cotyledons from the future plumule. The outer end of one of these divisions is marked by *x* in figures 52–56.

The next division in tier *b* is tangential, cutting off the dermatogen one stage earlier than in tier *a* (Fig. 52). The inner cells divide again longitudinally separating the future periblem from the future plerome (Fig. 53). Both longitudinal and transverse divisions occur in the future development of the periblem and plerome in this tier (Figs. 54–56). A heavy line separates the periblem from the plerome in figure 56.

Tier *c* develops into a single semi-circular layer of cells at the base of the embryo (Figs. 52–56). This layer helps to complete the periblem, dermatogen, and root cap of that region.

The single cell of tier *d* does not divide until the embryo is rather well developed; then it divides first by a transverse wall (Fig. 54). Both the proximal and distal cells from this division divide by longitudinal walls (Fig. 55). The cells of the proximal row do not divide again, but the cells of the distal row divide transversely (Fig. 56), separating a periblem portion from a dermatogen portion. The cells of the proximal row become a part of the root cap, which is increased on all sides by a single cell from tier *c* and in some places by extra cells cut off from the dermatogen of tier *b*. At the base of the mature em-

bryo there are one layer of root cap, one layer of dermatogen, and two layers of periblem (Fig. 56).

The basal cell of the suspensor extends into the micropylar haustorium and evidently functions as an embryonal haustorium (Figs. 44–50, 36–40). The two cells of the micropylar haustorium have grooves along their inner surfaces into which the embryonal haustorium fits (Fig. 42). The embryonal haustorium collapses before the seed is mature.

The development of the embryo as described here is in close agreement with the brief description given by Kausik (18) for *Lobelia trigona*. He did not make it clear where the three transverse divisions occur in the primary embryonal cell, and the plane of division for the first longitudinal division in cells *a* and *b* is not in agreement with that found in the present work. The development of the embryo as described by Armand (1) for three species of *Lobelia* agrees with the *Capsella*-type, which resembles the type discussed here only in the fact that it has a long suspensor. The differences between the two types of development lie in (1st.) the portion of the embryo derived from the distal cell following the first transverse division of the zygote, (2nd.) the formation of the octant stage, (3rd.) the plane of the first division in the distal cells of the octant stage, and (4th.) the amount of suspensor formed from the hypophysis. Cell *g* (Fig. 49) is considered the hypophysis in the present work.

The writer is of the opinion that there are as many examples showing some degree of relationship with the type of development described here as there are showing relationship with the *Capsella*-type. In very few papers are all of these points brought out, but some relationship is shown with this type of development in *Senecio aureus* described by Mottier (24); in *Erechtites hieracifolia* by Cooper (10); in *Silphium* by Merrell (23); in *Ulmus fulva* by Walker (40); in *Daucus carota* by Borthwick (4); in *Pontederia* by Smith (34); in *Rhytidophyllum* by Cook (7); in *Carum carvi* by Souèges (38); in *Sherardia arvensis* by Souèges (37); in *Myosurus minimus* by Souèges (35); in *Senecio* by Souèges (36); and in *Melilotus* by Cooper (8).

THE MATURE SEED

One layer of cells on the outside with a few extra cells around the haustoria make up the seed coat. The remainder of the integument has been absorbed by the endosperm. The cell walls of this outer layer have developed a thick band in the form of a ring on the four

lateral sides of the cell, touching neither the base nor the outer edge of the cell. A few of the outer cells at the micropylar end do not develop this thickened ring around the cell. The embryo is small, and most of the old suspensor is still attached to it. A large part of the mature seed is made up of endosperm, including the two big haustoria. The food material has been absorbed from the endosperm adjacent to the embryo, leaving around the embryo a large clear space which stains very little; the remainder of the endosperm stains readily (Fig. 43).

SUMMARY

1. In *Lobelia amoena* four microspores develop from a microspore mother cell, the cell plates forming after the second maturation division.

2. In the microspore, a small generative cell is cut off on one side, but later it moves into the tube cell where its cell membrane and then its cytoplasm disappear.

3. The tube nucleus disintegrates before the pollen tube begins to develop.

4. The generative nucleus divides in the tube to form the two male nuclei.

5. There are numerous anatropous ovules in each ovary.

6. A single hypodermal archesporial cell functions as the megaspore mother cell, usually producing four (sometimes three) megaspores, the chalazal one of which is functional.

7. The development of the embryo sac follows the monosporic 8-nucleate normal-type.

8. The antipodals begin disappearing by the time the sac is mature.

9. The mature synergids break through the micropylar end of the sac.

10. The single layer of nucellus breaks down following the first division of the functional megaspore.

11. The inner layer of cells of the massive integument develops into a conspicuous 'tapetum' which is present from the megaspore stage until the endosperm develops.

12. The polar nuclei fuse either before or during fertilization.

13. Double fertilization occurs.

14. Endosperm development is cellular; two cells from each end of the eight-celled endosperm develop into the large micropylar and chalazal haustoria.

15. The first division of the embryo cuts off a primary embryonal cell and a primary suspensor cell, the latter developing into an 8–12-

celled suspensor, the upper cell of which is embedded in the micropylar haustorium and functions as an embryonal haustorium.

16. The embryonal cell divides transversely, the proximal daughter cell dividing by two transverse walls forming four tiers of cells in the embryo which can be traced in the development to the mature embryo.

17. The octant stage is formed by two longitudinal divisions in both the distal and subdistal cells of the proembryo.

18. The first divisions in the four distal cells of the octant are anticlinal and separate the future cotyledons from the future plumule.

19. The first divisions in the four cells next to the distal tier of the octant stage cut off the dermatogen of that region, and the second divisions separate the future plerome from the future periblem.

20. All the derivatives from the primary embryonal cell develop into parts of the embryo; all the derivatives from the primary suspensor cell become part of the suspensor.

21. The seed coat consists of a single layer of cells whose lateral walls have become thickened.

22. Most of the mature seed is made up of endosperm, including the two large haustoria. The mature embryo is small.

LITERATURE CITED

1. ARMAND, L. Fécondation et développement de l'embryon chez les Lobéliacées. Compt. rend. Acad. Sci. **155**: 1534–1536. 1912.
2. BALICKA-IWANOWSKA, G. Contribution à l'étude du sac embryonnaire chez certains gametopétals. Flora **86**: 64–65. 1899.
3. BARNES, C. R. The process of fertilization in *Campanula americana* L. Bot. Gaz. **10**: 349–354. 1885.
4. BORTHWICK, H. A. Development of the macrogametophyte and embryo of *Daucus carota*. Bot. Gaz. **92**: 23–44. 1931.
5. BILLINGS, H. F. Beiträge zur Kenntniss der Samenentwickelung. Flora **88**: 304–309. 1901.
6. BUSHNELL, E. P. Development of the macrogametophyte in certain Labiatae. Bot. Gaz. **98**: 135–146, 190–197. 1936.
7. COOK, M. T. The embryology of Rhytidophyllum. Bull. Torrey Bot. Cl. **34**: 179–184. 1907.
8. COOPER, D. C. Macrosporogenesis and embryology of Melilotus. Bot. Gaz. **95**: 143–155. 1933.
9. COOPER, G. O. Cytological studies in the Chenopodiaceae. 1. Microsporogenesis and pollen development. Bot. Gaz. **97**: 169–178. 1935.
10. ——— Cytological investigations of *Erechtites hieracifolia*. Bot. Gaz. **98**: 348–355. 1936.
11. ——— Cytological investigations of *Pisum sativum*. Bot. Gaz. **99**: 584–591. 1938.

*12. COULTER, J. M., AND CHAMBERLAIN, J. M. Morphology of Angiosperms. D. Appleton & Co., New York. 1919.

*13. ELFVING, F. Studien über der Pollenkörner der Angiospermen. Jenaische Z. **13**: 1–28. 1879.

14. FINN, W. W. Spermazellen bei *Vinca minor* und *V. herbacea*. Ber. deutsch. bot. Ges. **46**: 235–246. 1928.

15. GUIGNARD, M. L. Recherches sur le sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. nat. Bot., Sér. 6, **13**: 136–199. 1882.

16. JOHANSEN, D. A. Studies on the morphology of the Onagraceae. Bot. Gaz. **90**: 75–91. 1930.

**17. JÖNSSON, J. Ytterligare bidrag till kännedomen om Angiospermernas embryosäckutveckling. Bot. Notiser. 1881.

18. KAUSIK, S. B. The life history of *Lobelia trigona* with special reference to the nutrition of the embryo sac. Proc. Indian Acad. Sci. Sect. B. **2**: 410–418. 1935.

19. LEE, A. B. The Microtomist's Vade-mecum. Tenth edition, edited by Gatenby and Painter. P. Blakiston's Son & Co. Philadelphia. 1937.

20. LOCKE, J. F. Microsporogenesis and cytokinesis in *Asimina triloba*. Bot. Gaz. **98**: 159–168. 1936.

21. MADGE, M. Spermatogenesis and fertilization in the cliestogamous flower of *Viola odorata* var. *praecox*. Ann. Bot. **43**: 545–577. 1929.

22. MAHESHWARI, P. A critical review of the types of embryo sacs in Angiosperms. New Phytol. **36**: 359–417. 1937.

23. MERRELL, W. D. A contribution to the life history of Silphium. Bot. Gaz. **29**: 99–133. 1900.

24. MOTTIER, D. M. On the embryo sac and embryo of *Senecio aureus* L. Bot. Gaz. **18**: 245–253. 1893.

25. OSTERWALDER, A. Beiträge zur Embryologie von *Aconitum napellus*. Flora **85**: 254–292. 1898.

26. PODDUBNAJA-ARNOLDI, W. A. Spermatogenesis bei einigen Compositen. Planta **4**: 284–298. 1927.

27. ——— Künstliche Kultur und zytologische Untersuchung des Pollenschlauches von *Senecio platanifolius*. Planta **19**: 299–304. 1933.

28. ROSEN, W. Zur Embryologie de Campanulaceen und Lobeliaceen. Acta Horti Gothoburgensis **7**: 31–42. 1932.

29. SAX, K., AND EDMONDS, H. W. Development of the male gametophyte in Tradescantia. Bot. Gaz. **95**: 156–163. 1933.

***30. SCHLOTTERBECK, J. O. Beiträge zur Enwickelungsgeschichte phamakognostisch wichtiger Samen. Diss. Bern. 1896.

31. SCHNARF, K. Vergleichende Embryologie der Angiospermen. Berlin. 1931.

32. SCHURHOFF, P. N. Die Zytologie der Blutenpflanzen. Stuttgart. 1926.

33. SHATTUCK, C. H. A morphological study of *Ulmus americana*. Bot. Gaz. **40**: 209–223. 1905.

* Citation from Wulff and Maheshwari (43).

** Citations from Schnarf (31) and Schurhoff (32).

*** Citation from Balicka-Iwanowska (2).

34. SMITH, W. R. A contribution to the life history of Pontederiaceae. Bot. Gaz. **25**: 324–337. 1898.
35. SOUÈGES, R. Recherches sur l'embryogénie des Renonculacées. Bull. Soc. Bot. Fr. **58**: 629–636. 1911.
36. ———. Embryogénie des Composées. Compt. rend. Acad. Sci. **171**: 254–256, 356–357. 1920.
37. ———. Développement de l'embryon chez le *Sherardia arvensis* L. Bull. Soc. Bot. Fr. **72**: 546–564. 1925.
38. ———. Recherches sur l'embryogénie des Ombellifères. Bull. Soc. Bot. Fr. **77**: 494–511. 1930.
39. VESQUE, J. Développement du sac embryonnaire des Phanérogames Angiospermes. Ann. Sci. nat. Bot., Sér. 6, **6**: 237–285. 1878.
40. WALKER, R. I. Macrosporogenesis and embryo development in *Ulmus fulva*. Bot. Gaz. **99**: 592–598. 1938.
41. WARD, M. H. A contribution to our knowledge of the embryo-sac in Angiosperms. Journ. Linn. Soc. Bot. London **17**: 519–546. 1880.
42. WARMING, E. De l'ovule. Ann. Sci. nat. Bot., Sér. 6, **5**: 177–266. 1878.
43. WULFF, H. D., AND MAHESHWARI, P. Male gametophyte in Angiosperms (A critical review). Jour. Indian Bot. Soc. **17**: 117–140. 1938.
44. WYLIE, R. B. Sperms of *Vallisneria spiralis*. Bot. Gaz. **75**: 191–202. 1923.

EXPLANATION OF THE PLATES

The figures are arranged so that the micropylar end of the ovule is turned towards the bottom of the page.

PLATE 10

Fig. 1. Cross section of a young anther showing four layers of wall cells surrounding a mass of microspore mother cells. × 780.

Fig. 2. Microspore mother cell in early prophase of first maturation division. × 780.

Fig. 3. Microspore dyad, just after first maturation division. × 780.

Fig. 4. Three of the four microspore nuclei just after the second maturation division. × 780.

Fig. 5. Wall formation between the microspores after the second maturation division. × 780.

Fig. 6. Young microspore, freed from mother cell, showing a thin band of cytoplasm surrounding a large central vacuole. × 780.

Fig. 7. Slightly older microspore with a number of small vacuoles instead of the one large vacuole. × 780.

Fig. 8. Microspore with generative and tube cells. × 975.

Fig. 9. Generative cell surrounded by cytoplasm of tube cell. Part of the contents of the generative cell do not stain. The tube nucleus is beginning to disintegrate, i.e. the nuclear membrane is breaking down. × 780.

Fig. 10. Mature pollen grain showing three pores in the exine. The generative nucleus is surrounded by a small mass of deeply staining cytoplasm. There are three small bodies in the cell which appear as the tube nucleus disappears. × 780.

Fig. 11. Microspore with young pollen tube, oblong generative nucleus. × 780.
Fig. 12. More mature pollen tube showing generative nucleus in the tube. × 780.
Fig. 13. Portion of mature pollen tube showing two male nuclei. × 780.
Fig. 14. Longitudinal section of a young ovule showing a single hypodermal archesporial cell, a single layer of nucellus, the beginning of a single integument, and a funiculus. × 780.
Fig. 15. Longitudinal section through an older ovule, showing mature megaspore mother cell surrounded by nucellus and integument. × 780.
Fig. 16. Longitudinal section through nucellus, showing dyad formed during first maturation division of megaspore mother cell. × 780.
Fig. 17. Longitudinal section through nucellus and portion of surrounding integument showing four linear megaspores and the beginning of a 'tapetum.' × 780.

PLATE 11

Fig. 18. Similar to Fig. 17, showing chalazal megaspore developing at the expense of the other three. × 780.
Fig. 19. Similar to Fig. 17. In this case the nuclear division of the micropylar cell of the dyad was not followed by cytoplasmic division. There are only two megaspores to disintegrate. × 780.
Fig. 20. Older stage, same plane, showing three collapsed micropylar megaspores, and one large chalazal megaspore with a vacuole at each end. × 780.
Fig. 21. Longitudinal section through megaspores showing the second and fourth megaspores, counting from the micropylar end, developing into embryo sacs while the first and third are disintegrating. × 780.
Fig. 22. Longitudinal section through ovule showing two-nucleate embryo sac already broken through the nucellus at the micropylar end, showing a vacuole in the center of the sac, a large integument, and a well developed 'tapetum' surrounding the nutritive tissue at the chalazal end of the ovule. × 388.
Fig. 23. Two-nucleate embryo sac similar to one in figure 22. × 780.
Fig. 24. Longitudinal section of four-nucleate embryo sac. A small vacuole has appeared at the chalazal end of the sac. × 780.
Fig. 25. Longitudinal section of eight-nucleate sac, with small vacuole at each end of the sac. × 780.
Fig. 26. Longitudinal section of an older ovule showing a well developed integument and 'tapetum,' the old nucellus, three well developed antipodal cells, two polar nuclei, one synergid, and an egg cell. The other synergid was cut off in the adjacent section. × 337.
Fig. 27. Two polar nuclei touching. × 780.
Fig. 28. Two polar nuclei in the process of fusion; the nucleoplasm has fused, but the nucleoli have not. × 780.
Fig. 29. Mature embryo sac ready for fertilization, showing two synergids, egg cell, fusion nucleus, and three disintegrating antipodal cells. × 780.
Fig. 30. Cross section of synergids above their nuclei. × 780.
Fig. 31. Triple fusion of two polar nuclei and male nucleus. × 780.
Fig. 32. Egg nucleus and male nucleus uniting; the male nucleus is smaller and stains lighter. × 780.

PLATE 12

Fig. 33, 34. Male nucleus uniting with fusion nucleus. The nucleoli may remain distinct for some time after the nucleoplasm fuses. × 780.

Fig. 35. Longitudinal section through the embryo sac just after fertilisation, showing the first division of the endosperm nucleus; the egg cell, the nucleus of which contains the nucleoli of the egg and male nucleus, the nucleoplasm already fused; the pollen tube, which has destroyed one of the synergids; and the other synergid still in a normal condition. × 780.

Fig. 36. Longitudinal section through an older ovule, showing 'tapetum'; four cells in the endosperm, the two micropylar cells pushing up around and beyond the zygote, which has elongated but not divided; the nucleoli still distinct in the zygote nucleus. × 337.

Fig. 37. Section showing transverse division of cells in the four-celled endosperm. × 337.

Fig. 38. Similar to fig. 36, slightly older, showing micropylar haustorium forming from two micropylar cells of the endosperm, embryonal haustorium within the micropylar endosperm haustorium, the embryonal haustorium at this stage being merely the micropylar end of the much elongated zygote; and a chalazal haustorium developing from two chalazal cells of the endosperm at the expense of the nutritive tissue in the chalazal end of the ovule. × 172.

Fig. 39, 40. Longitudinal sections of older ovules showing the elongation of the ovule accompanied by the multiplication of the cells of the endosperm and the stretching of the cells of the integument; the enlarging haustoria; and the developing embryo. × 172.

PLATE 13

Fig. 41. Cross section of chalazal haustorium. × 172.

Fig. 42. Cross section of micropylar haustorium. × 172.

Fig. 43. Longitudinal section of mature seed, showing seed coat made up of scarcely more than a single layer of cells; the massive endosperm still attached to the two haustoria; the embryo with suspensor collapsing. The embryo has used the food material in the endosperm cells all around it. × 78.

Fig. 44–56. Longitudinal sections of embryos. For explanations see the text. Figure 56 is a section of a mature embryo. × 337.

PLATE 10

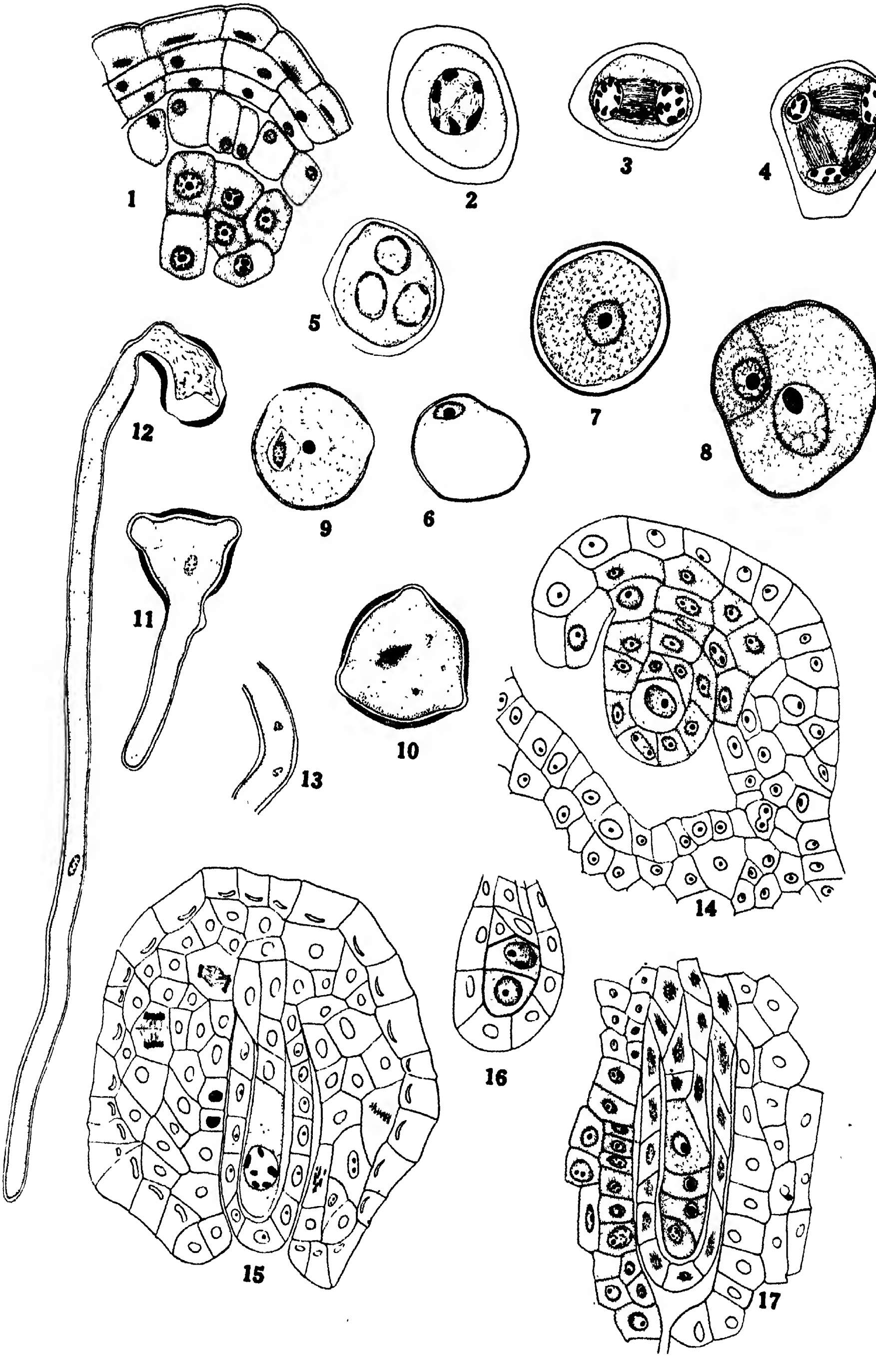


PLATE 11

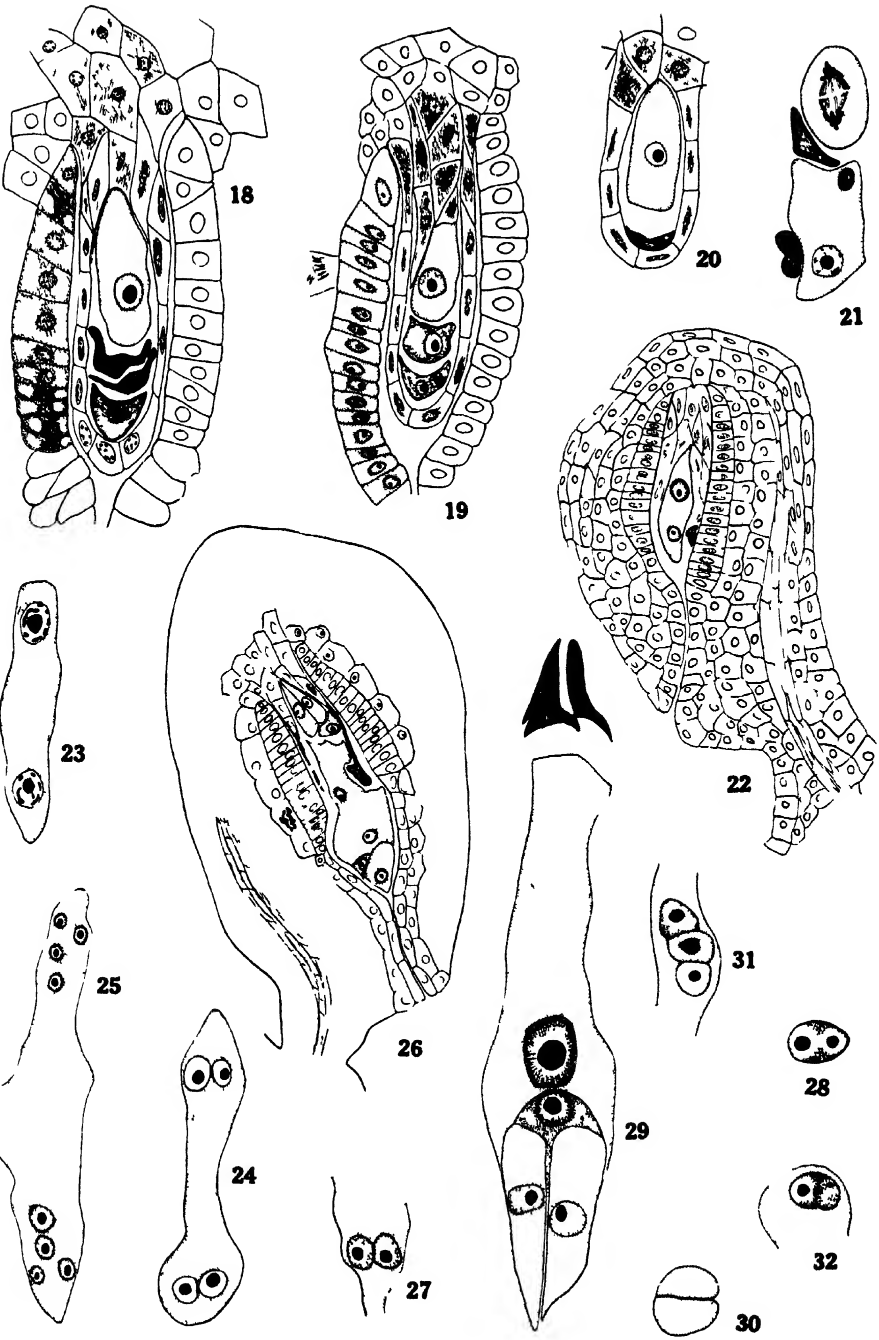
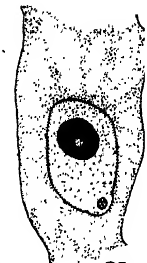
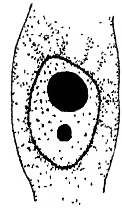
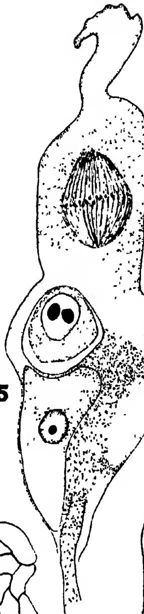
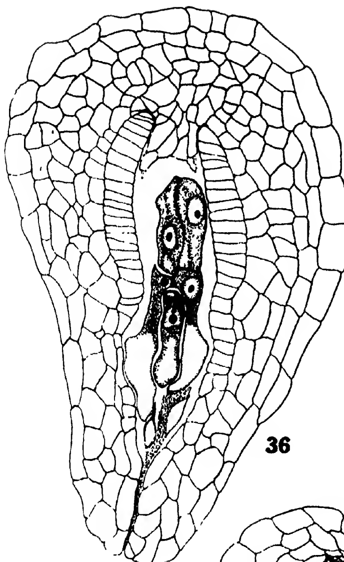


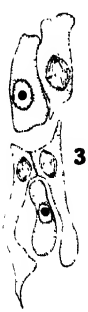
PLATE 12

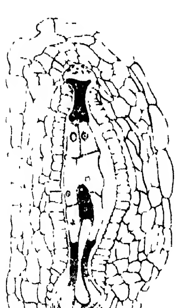

33

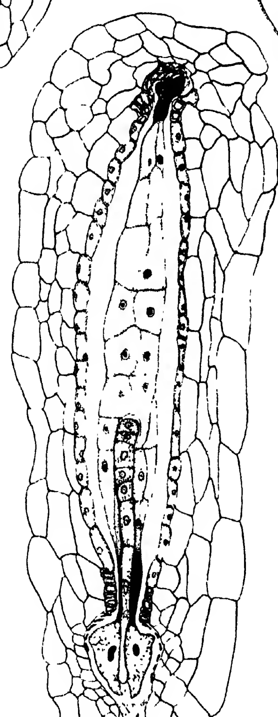

34


35


36


37


38


39

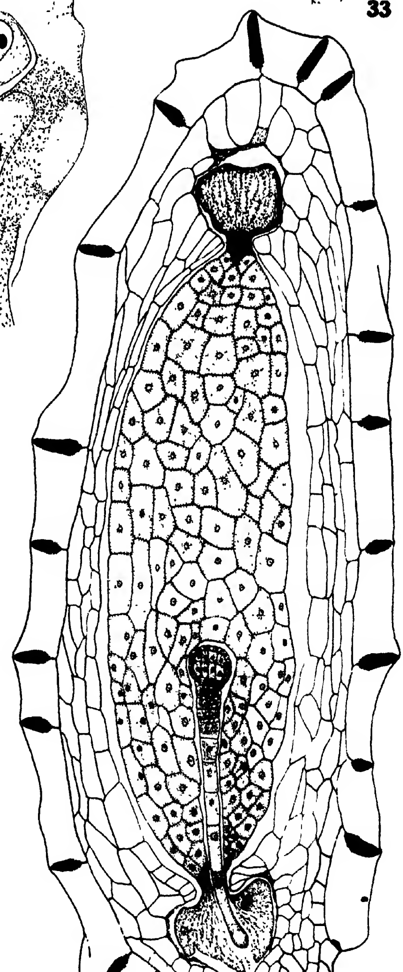
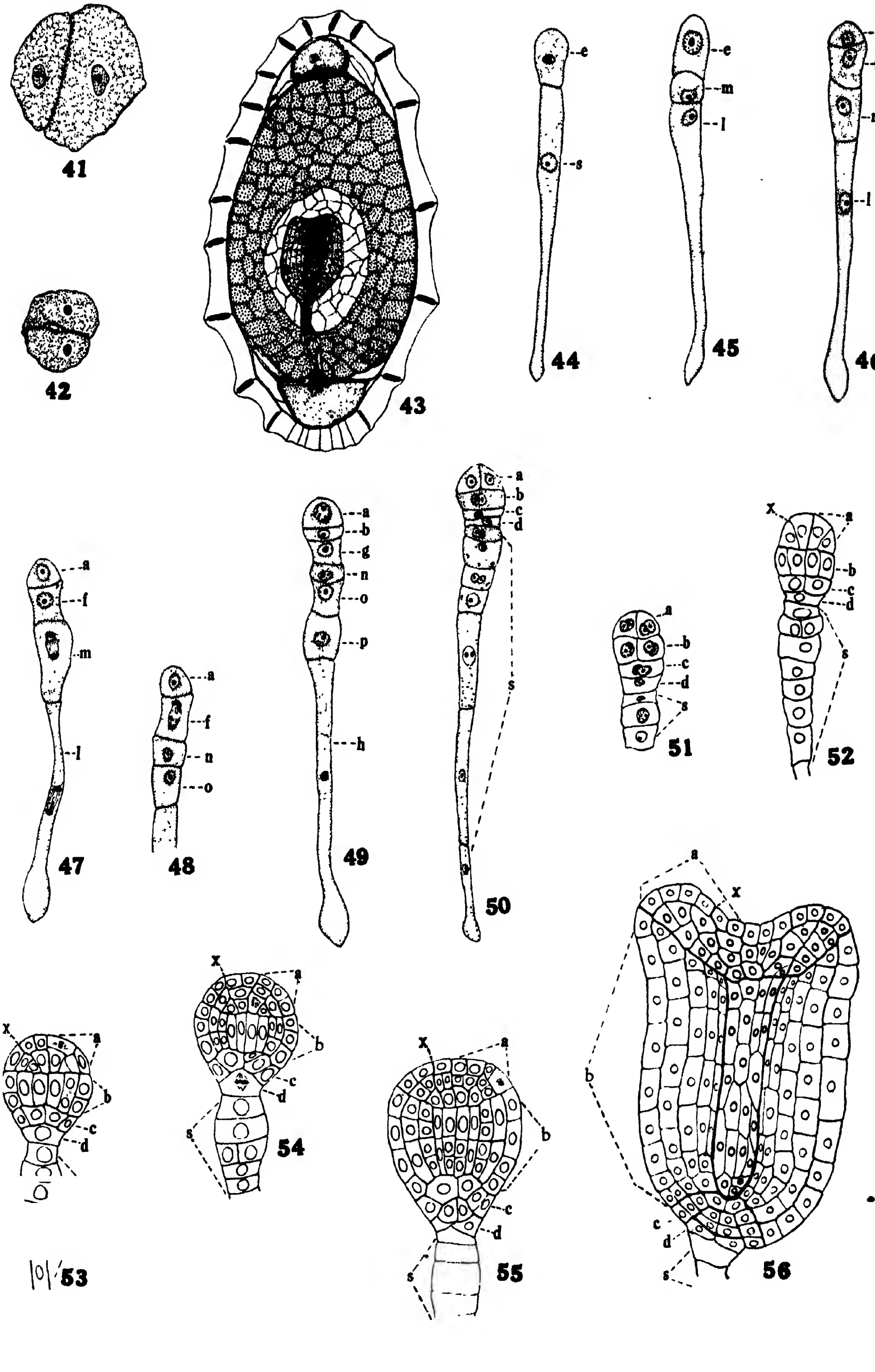

40

PLATE 13



FRESHWATER ALGAE OF NORTH AND SOUTH CAROLINA. PART I. CYANOPHYCEAE*

By Paul J. Philson

Plates 14–17

No systematic study of the blue-green algae of North and South Carolina has ever been attempted. Ravenel in 1869 and 1877, Wolle in 1887, and Green in 1897 have made known the only identifications from these states. The lists submitted by these men are much too meager to give any idea of the relative abundance of the species of this class. Ravenel has identified 14 species from South Carolina, Wolle 2 species, and Green 3 species. Ravenel and Green have published 1 species each from North Carolina. These 21 species constitute the recorded blue-green flora of this region up to the present time. Unfortunately the writer has had no opportunity to examine the specimens of the above collectors.

It is interesting to note that no species of the families Chroococcaceae, Chamaesiphonaceae, or Rivulariaceae has ever been recorded.

Systematic work on the blue-green algae was begun in North Carolina in the fall of 1930. The boundaries were later extended to include South Carolina as well. Samples of blue-green algae were collected from time to time from these states until the spring of 1934. The submitted list of species is far from being complete, but it probably contains at least the more common forms.

The writer wishes to express his thanks to Miss Josephine E. Tilden, former Professor of Botany at the University of Minnesota, under whose able guidance this work was accomplished, and to Professor E. L. Green, of the Ancient Language Department at the University of South Carolina, for having supplied the Latin descriptions for new species.

METHOD

The method of collecting and preserving Cyanophyceae for future identification is simplified considerably because of the hardiness of these

* A doctoral thesis submitted to the Graduate Faculty of the University of Minnesota.

plants and because they do not plasmolize readily. A 5% solution of formaldehyde proved to be the best fixing and preserving agent. In this solution, the plants keep for an indefinite period of time.

Upon being collected in the field, the material was placed in small vials containing the fixing solution. Those intended for immediate study were brought into the laboratory in water.

For identification purposes, the material is best studied from semi-permanent mounts, preferably glycerine mounts. Glycerine not only holds the plants in a stationary position, but also causes the trichomes to become more transparent. To make these mounts a small sample of the material is taken from the formaldehyde solution and thoroughly washed in water. It is then transferred to a 10% glycerine solution and exposed to the air to permit evaporation. When the glycerine has become sufficiently concentrated, the plant mass is gently teased apart and portions are transferred onto a slide in a drop of pure glycerine. A cover glass is added and the material is ready for study. The mount is less likely to be destroyed if sealed with balsam, or some similar substance.

With unicellular forms, the washing process may be omitted as many plants are likely to be lost. The small amount of formalin remaining in the glycerine does not noticeably affect the plants.

The accompanying drawings, all of which are original, were made by the method employed in the phycological laboratories of the University of Minnesota (see Tilden in bibliography).

CLASSIFICATION

The system of classification here adopted is essentially that which has been in general use since the latter part of the nineteenth century. Minor differences will be observed, however.

Order I. COCCOGONALES. Plants unicellular, single or associated in colonies which are usually embedded in a mucous integument, never filamentous; reproduction usually by simple division of the parent cell into two daughter cells, more rarely by the formation of "endogonidia."

Family 1. CHROOCOCCACEAE. Plants unattached, showing no differentiation between base and apex; reproduction by division in one, two, or three planes.

Family 2. CHAMAESIPHONACEAE. Plants attached, showing a decided differentiation between base and apex; reproduction by the formation of "endogonidia."

Order II. HORMOGONALES. Plants multicellular and filamentous; filaments branched or unbranched, consisting, usually, of one or more rows of cells surrounded by a sheath; reproduction by means of hormogones or hypnogonidia.

Family 3. OSCILLATORIACEAE. Plants simple or branched; sheaths variable, invisible or distinct; trichomes simple, consisting of a single row of vegetative cells having a constant diameter except at the apex; heterocysts absent; reproduction by the formation of hormogones.

Family 4. PLECTONEMATACEAE. Filaments in free-floating caespitose masses or densely interwoven to form felts or mats; sheaths firm, rather thin, sometimes lamellose, hyaline or rarely yellow-brown; false branches frequent, usually in pairs; trichomes fitting closely within the sheaths, often slightly constricted between the cells; heterocysts absent; reproduction by hormogones.

Family 5. NOSTOCACEAE. Individual sheaths mostly indistinct, confluent; trichomes usually much twisted and contorted, consisting of vegetative cells, heterocysts, and often hypnogonidia; reproduction by the formation of hormogones and hypnogonidia.

Family 6. SCYTONEMATACEAE. Filaments exhibiting false branching; sheaths firm, homogeneous or variously layered; trichomes consisting of a single row of vegetative cells and heterocysts; reproduction by the formation of hormogones, rarely by hypnogonidia.

Family 7. STIGONEMATACEAE. Filaments exhibiting true branching; sheaths mostly wide, sometimes very irregular; trichomes consisting of one to several rows of cells; heterocysts usually scarce and poorly developed; reproduction by the formation of hormogones, hormocysts, and hypnogonidia.

Family 8. RIVULARIACEAE. Filaments exhibiting false branching; sheaths usually firm and distinct, occasionally indistinct; trichomes tapering from base to apex, usually ending in a long multicellular hair; heterocysts mostly basal; reproduction by the formation of hormogones and hypnogonidia.

Order COCCOGONALES Atkinson, 1905

Unicellular plants, single or in colonies, rarely arranged in filaments; vegetative reproduction by division in single cells.

Family CHROOCOCCACEAE Naegeli, 1849

Reproduction entirely by cell division (fission) in one, two, or three planes at right angles to one another; plants showing no distinction between basal and apical regions.

a. Plants solitary or associated in small, individual families or colonies, not surrounded by a common gelatinous tegument *Chroococcus*
aa. Plants associated in families or colonies, surrounded by a common gelatinous tegument.
  b. Cells aggregated to form gelatinous colonies of no definite shape.
    c. Individual sheaths distinct, thick, vesicle-like *Gloeocapsa*
    cc. Individual sheaths not distinct.
      d. Cells spherical; cell divisions in all directions *Aphanocapsa*
      dd Cells oblong to cylindrical; cell division in one direction *Aphanothece*
  bb. Cells aggregated to form gelatinous colonies of a definite, characteristic shape.
    c. Cells pear-shaped or heart-shaped, terminating mucous stalks radiating from the center of the colony *Gomphosphaeria*
    cc. Cells spherical, mucous stalks absent *Microcystis*

CHROOCOCCUS Naegeli, 1849

Plants spherical or hemispherical after division, with homogeneous or lamellated individual sheaths, solitary or in very small colonies; colonies free-floating or forming a stratum in damp places.

Chroococcus turgidus (Kuetz.) Naeg. Gattungen einzelliger algen, p. 46. 1849. (*Protococcus turgidus* Kuetz. Tab. phycol. **1**: 5, tab. 6. 1845.) *Fig. 12.*

The colonies were nearly spherical in shape, and were composed of from two to four cells. They measured 18.6 to 65 mic. in diameter. The sheaths were wide, lamellose, and a few were yellow in color. Cell diameters varied from 12.3 to 27, rarely 38 mic.

Hollow Rock, Durham County, N. C.; Alligator Lake, near Myrtle Beach, Horry County, S. C.

GLOEOCAPSA Kuetzing, 1843

Plant mass forming more or less expanded gelatinous crusts or strata on wet or dripping rocks or in damp places; plants spherical, each consisting of a cell inclosed in a vesicle-like, strongly thickened, homoge-

neous or stratified, colorless or colored mucilage sheath, associated in groups of from 2 to 32, equally distributed throughout the colony but always some distance from one another.

a. Sheaths thick, colorless; plants 7 to 11 mic. in diameter; cells 3 to 6 mic. in diameter..*G. conglomerata*
aa. Sheaths indistinct, yellowish or brownish; plants 12 mic. in diameter; cells 3 to 4.5 mic. in diameter..............................*G. sparsa*

Gloeocapsa conglomerata Kuetz. Tab. phycol. **1**: 16, pl. 20. 1845–1849. *Fig. 3.*

Several colonies studied varied from 20 to 44 mic. in diameter. The plants were from 6 to 10 mic. in diameter, with very wide sheaths. At times, many cells were aggregated within a single sheath. Cells measured from 3.4 to 4.9 mic.

Associated with other algae on damp ground in the greenhouse of Duke University, Durham, N. C.

Gloeocapsa sparsa Wood. Proc. Amer. Phil. Soc. **11**: 123. (1869) 1871.

Colonies were somewhat globose with wide, lamellose and yellowish sheaths. The cells measured 3 to 4.8 mic., and possessed finely granular contents.

Hollow Rock, Durham County, N. C. Growing on wet dripping rocks with species of *Stigonema* and *Scytonema*.

APHANOCAPSA Naegeli, 1849

Plants forming colonies of no definite shape; individual sheaths not distinct, confluent with the colonial envelope; cells spherical.

Aphanocapsa grevillei (Hassall) Rabenh. Flora europaea algarum **2**: 50. 1865. (*Coccochloris grevillei* Hassall. A history of the British freshwater algae, p. 318, pl. 78, fig. 7, a–b, and 8. 1845.) *Fig. 4.*

Colonies of this species were numerous in a mass of algae which covered the bottom of a lake to a depth of from 6 to 12 inches. The globose plant masses measured about 49 mic. in diameter, while the cells measured 3.2 to 5.8 mic.

Hollow Rock, Durham County, N. C.; Alligator Lake, near Myrtle Beach, Horry County, S. C.

APHANOTHECE Naegeli, 1849

Plants oblong to cylindrical, distributed throughout the colony in a solid mass; individual sheaths not distinct, confluent with the colonial envelope; colonies somewhat globose or without definite shape, sometimes endophytic.

a. Plant mass colorless .... *A. microscopica*
aa. Plant mass colored.
b. Plant mass dirty-green or yellowish-brown; cells 2.5–3 x 4.5–5.5 mic., thickly aggregated within the colony .... *A. conferta*
bb. Plant mass pale blue-green; cells 3–8 x 4.5–24 mic., usually scattered *A. pallida*

Aphanothece microscopica Naeg. Gattungen einzelliger algen, p. 59, pl. 1, H, fig. 1. 1849. *Fig. 1.*

The colonies were somewhat young since all were globose to oval in shape. They measured about 40 mic. in diameter. The cells were 3.8–4.4 x 3.8–8.2 mic., and were nearly spherical immediately after division.

Alligator Lake, Horry County, S. C. The very abundant colonies were floating in a mass of mostly unicellular algae that covered the bottom of the lake to a depth of from six to twelve inches.

Aphanothece conferta Richter. In Hauck and Richter, Phykotheka Universalis, no. 487. 1892.

The cells, which were thickly grouped together in a colorless expanded mass, measured 1.2–2.9 x 3–5.4 mic., a little smaller than the type for this species.

Durham, Durham County, N. C. Forming olive-green, expanded, gelatinous masses on the concrete wall of the aeration pool in refrigerating plant of the Duke University Hospital.

Aphanothece pallida (Kuetz.) Rabenh. Fl. eur. algarum 2: 64. 1865. (*Palmella pallida* Kuetz. Phycol. germanica, p. 149. 1845.) *Fig. 11.*

Several *Aphanothece*-like cells were scattered in the gelatinous matrix of other algae. They measured 4.5–6.1 x 4.8–8.2 mic. The cells were too few to allow positive identification.

Durham, Durham County, N. C. Growing in a gelatinous plant mass in a wet corner of the Duke University greenhouse.

GOMPHOSPHAERIA Kuetzing, 1836

Plants forming solid, free-floating, spherical or ellipsoid colonies, the cells generally lying some distance from one another and forming a layer one cell in thickness toward the periphery of the colonial envelope, joined at base to branched mucilage stalks, which are formed from the remnants of the parent cell membranes; colonies embedded in a copious, hyaline, homogeneous, gelatinous envelope; individual sheaths distinct or confluent with the colonial envelope; cells wedge-shaped, pear-shaped, or heart-shaped.

Gomphosphaeria aponina Kuetz. var. **delicatula** Virieux. Rech. lac Jura. Ann. biol. lac. **8:** 1916. *Fig. 10.*

These colonies varied from 30 to 85 mic. in diameter and were provided with glistening, homogeneous, hyaline, sometimes yellowish teguments. The cells measured 3–5 x 5.5–8.9 mic. While a few of the cell measurements seem to coincide with those of the typical species, the greater number falls within the limits of the variety.

Alligator Lake, Horry County, S. C. Numerous colonies were scattered throughout an expanded mass of algae that covered the bottom of the lake to a considerable depth.

MICROCYSTIS Kuetzing, 1833

Plants forming solid, free-floating, globose, elongated, or clathrate colonies; individual sheaths confluent with the colonial envelope; cells spherical.

a. Cells without pseudovacuoles, 2–3 mic. in diameter........ ...*M. pulverea*
aa. Cells with pseudovacuoles, 3–7 mic. in diameter...........*M. aeruginosa*

Microcystis pulverea (Wood) Forti. In De Toni, J. B. Sylloge algarum **5:** 92. 1907. (*Pleurococcus pulvereus* Wood. Smith. Contrib. to Knowledge **19** (No. 241): 79. 1872.)

The pulverulent colonies were spherical or oval in shape and the hyaline teguments were observed only occasionally. The cells, whose contents were homogeneous, measured 1.8 to 3.2 mic. in diameter, with a few whose diameters coincided with those of variety *incerta* (Lemm.) Crow.

Alligator Lake, Horry County, S. C. In a mass of algae covering the bottom of the lake to a depth of from six to twelve inches; the whitish blue-green colonies not very abundant.

Microcystis aeruginosa Kuetz. Tab. phycol. **1:** 6, tab. 8. 1845. *Fig. 2.*

Globose and clathrate colonies, which were microscopic in size, were found among filaments of *Oedogonium* sp. The spherical cells measured 3–5.5 mic. in diameter and contained numerous pseudovacuoles.

Durham, Durham County, N. C. In a small temporary stream in Duke Forest, near Duke University.

Family CHAMAESIPHONACEAE Geitler, 1925

Plants erect, cylindrical, epiphytic; reproduction by gonidia formed at the apex of the cell by abstriction; plants showing a differentiation between base and apex.

CHAMAESIPHON A. Braun and Grunow, 1865

Plants epiphytic, unicellular, solitary or aggregated in dense clusters, ovoid, pyriform, cylindrical or much elongated, attached at base, often widening upward to free apex; sheath thin, ruptured at apex; reproduction by gonidia which are abstricted successively from the upper portion of the protoplast.

Chamaesiphon incrustans Grunow. In Rabenhorst, Fl. eur. algarum **2:** 149. 1865. *Fig. 7.*

The plants, abundant on a few filaments of *Oedogonium* sp., were 4–6.3 x 5–24 mic. in size. The cell contents were pale blue-green and homogeneous.

Durham, Durham County, N. C. Attached to filaments of *Oedogonium* sp. from a brook in the Duke Forest.

Order HORMOGONALES Atkinson, 1905

Multicellular plants, either single or in colonies, distinctly filamentous; vegetative reproduction by groups of cells, numbering two to many, termed hormogones.

Family OSCILLATORIACEAE Harvey, 1858

Filaments simple or branched, containing one or more trichomes; sheaths variable, more or less gelatinous; trichomes consisting of a simple row of cells uniform along their entire length, except for the apical cells which sometimes taper more or less; reproduction by means of hormogones.

a. Sheaths not or scarcely visible............................*Oscillatoria*
aa. Sheaths evident, very thin in some species.
b. Sheaths open at the extremities, containing only a single trichome.
c. Filaments partially agglutinated; sheaths thin, more or less confluent.......................................*Phormidium*
cc. Filaments free; sheaths thick, firm, cylindrical........*Lyngbya*
bb. Sheaths usually closed at the extremities; containing two to many trichomes.
c. Prostrate sheaths mucous, homogeneous, colorless...*Microcoleus*
cc. Sheaths firm, more or less lamellose, hyaline or colored *Schizothrix*

OSCILLATORIA Vaucher, 1803

Trichomes simple, free, often forming dense tangled masses, cylindrical, smooth or constricted at joints, not moniliform, straight or bent, not spirally twisted; apices straight, bent, or hooked, more or less tapering; apical cell simple, calyptrate (provided with a thickened membrane), or capitate (exhibiting a "head").

a. Cells 2–10 mic. in diameter, longer than ⅓ their diameter.
b. Cells 4–10 mic. in size, constricted at joints; apical cell not capitate *O. tenuis*
bb. Cells 2–3 mic. in size, not constricted at joints; apical cell capitate *O. splendida*
aa. Cells 11–60 mic. in diameter, very short, not longer than ⅓ their diameter.
b. Cells 11–20 mic. in size, not capitate; cross walls granular. .*O. limosa*
bb. Cells 16–60 mic. in size, somewhat capitate; cross walls not granular *O. princeps*

Oscillatoria tenuis Agardh. Algarum decades **2**: 25. 1813. *Fig. 8.*

The trichomes, in the material studied, varied in diameter from 6.1 to 7.3 mic. The cells measured from 2.9 to 4.1 mic. in length, with the transverse walls distinctly granular.

Durham, Durham County, N. C.

Oscillatoria splendida Greville. Flora Edinensis, p. 304. 1824.

The gradually tapering trichomes and the apically inflated cell are characteristic of the species.

Hollow Rock, Durham County, N. C. Intermixed with *Lyngbya martensiana* Meneghini from a small stream.

Oscillatoria limosa (A. G. Roth) Agardh. Dispositio algarum sueciae, p. 35. 1812. (*Conferva limosa* Roth. Catalecta botanica **3**: 197. 1797–1806.)

The trichomes were 12.3 to 14.3 mic. in diameter. Cell lengths varied from 1.7 to 3.5 mic. All transverse walls were granulated.

Old Fort, McDowell County, N. C. The plants were intermixed with *Anabaena laxa* (Rabenhorst) A. Braun, together forming a thin, blue-green coating on the bottom of Mill Creek four inches below the surface.

Oscillatoria princeps J. P. Vaucher. Histoire des Conferves d'eau douce, p. 190, pl. 15, fig. 2. 1803.

The floating plant masses were variable in size from two to twelve square inches, and were of a bright blue-green color. All of the measurements were within the limits 41–43 x 4.1–6.1 mic. No cells were constricted at the joints.

Lake Junaluska, Haywood County, N. C. The plants were matted together in large masses which floated during the hot part of the day, sinking to the bottom of the lake at night.

PHORMIDIUM Kuetzing, 1843

Filaments simple, agglutinated, forming a woolly or felt-like layer or rarely floating, attached at the base, with free ends torn and ragged; sheaths thin, transparent, mucous, agglutinated, partly or entirely diffluent; trichomes cylindrical, in some cases constricted at joints; apex of trichome often tapering, straight or curved, capitate in some species; apical cell sometimes calyptrate, or capitate.

a. Apex of trichome straight or curved, capitate.
  b. Apex of trichome straight .... *P. favosum*
  bb. Apex of trichome curved....... .... *P. autumnale*
aa. Apex of trichome always straight, not capitate.
  b. Trichomes tapering toward the apices.
    c. Cells 3–4.5 x 3.4–8 mic.; apical cell obtuse-conical. . . *P. corium*
    cc. Cells 7.5–10.5 x 4–8 mic.; apical cell truncate...... . *P. crouani*
  bb. Trichomes not tapering toward the apices.
    c. Trichomes moniliform; cells 0.6-0.8 x 1.2–6.4 mic. *P. angustissimum*
    cc. Trichomes not moniliform; cells 4.5–12 x 4–9 mic....... *P. retzii*

Phormidium favosum (Bory) Gomont. Monog. des Oscillariées, etc., p. 200, pl. 5, fig. 14–15. 1893. (*Oscillaria favosa* Bory. Dict. classique hist. nat. **12**: 466, pl. 12. 1827.)

The gradually tapering, straight, capitate apex of the trichome is characteristic for the species. In the plants studied, the diameter of the cells was 4.5 mic., the minimum for this species.

Durham, Durham County, N. C. Forming a thin coating on wet rocks, west campus of Duke University.

Phormidium autumnale (Agardh) Gomont. Monog. des Oscillariées, etc., p. 207, pl. 5, fig. 23–24. 1893. (*Oscillatoria autumnale* Agardh. Dispositio algarum sueciae, p. 36. 1812.) *Fig. 9.*

The deep blue-green mats formed tufts of wide extent, a single tuft being composed of many filaments whose sheaths, for the most part, had become diffluent. The lengths of the cells were a little less than for those of the species, being 3–4.1 mic., but all other characters were in agreement with the species.

Charlotte, Mecklenburg County, N. C. Forming a thin, blue-green coating on submerged rocks in a narrow creek near the city limits.

Phormidium corium (Agardh) Gomont. Monog. des Oscillariées, etc., p. 192, pl. 5, fig. 1–2. 1893. (*Oscillatoria corium* Agardh. Dispositio algarum sueciae, p. 36. 1812.)

The material studied grew in irregular, thin, slimy, light green patches on wet clay. The cells measured 4.5–9 mic. in length. No cyanophycin granules were present, but the contents of the cells were finely granular.

Durham, Durham County, N. C. Forming close, felt-like, irregular patches on the bottom of a rain pool on the campus of Duke University.

Phormidium crouani Gomont. Monog. des Oscillariées, etc., p. 195, pl. 5, fig. 5. 1893. *Fig. 5.*

The filaments formed widely expanded, mucilaginous, irregular, blue-green masses. Cell measurements fell within the limits 7.4–8.3 x 4.1–8.2 mic. The contents of the cells were finely granular and the transverse walls very distinct.

Hollow Rock, Durham County, N. C. Growing in patches on water-soaked soil.

Phormidium angustissimum W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35:** 298. 1897.

Its minute size and the moniliform nature of the trichome are features characteristic for the species.

Forming a thin blue-green coating on the bottom of a small creek at Hollow Rock, Durham County, N. C.

Phormidium retzii (Agardh) Gomont. Monog. des Oscillariées, etc., p. 195, pl. 5, fig. 6–9. 1893. (*Oscillatoria retzii* Agardh. Dispositio algarum sueciae, p. 36. 1812.) *Fig. 6.*

The plants grew in irregular, dull green, coarse masses. Most of the sheaths were diffluent. The trichomes, which were steel blue-green in color, were long and straight, the apical cell lacking a calyptra. The cells measured 5.3–6.1 x 4.5–9 mic. Their contents were finely granular.

Durham, Durham County, N. C. Growing intermixed with *Vaucheria* sp. two inches above the water level on the wet bank of a small stream.

LYNGBYA C. A. Agardh, 1824

Filaments free or aggregated into dense, floccose, caespitose masses, simple, or very rarely branched in some species, attached or free-floating; sheaths firm, of varying thickness, thin and homogeneous or thick and lamellated, colorless or rarely yellowish-brown; trichomes not constricted at the dissepiments, tapering at the apex only slightly or not at all; wall of apical cell sometimes thickened, forming a calyptra.

a. Cells 4–6 mic. in diameter........................*L. aerugineo-caerulea*
aa. Cells 6–28 mic. in diameter.
  b. Cells 6–10 x 1.7–3.3 mic.; apical cell not capitate; sheaths colorless *L. martensiana*
  bb. Cells 8–28 x 2.7–5.6 mic.; apical cell capitate; sheaths becoming yellowish or brownish..............................*L. aestuarii*

Lyngbya aerugineo-caerulea (Kuetz.) Gomont. Monog. des Oscillariées, etc., p. 166, pl. 4, fig. 1–3. 1893. (*Oscillaria aerugineo-caerulea* Kuetz. Phycol. generalis, p. 185. 1843.)

Most of the plant masses were dark blue-green and slimy to the touch. The sheaths were colorless and somewhat firm, and the cells, with granular contents, were blue-green in color. The latter measured 3.9–5.9 x 2.1–3.6 (rarely 5.4) mic.

Durham, Durham County, N. C. Forming slimy masses about one square inch in area on the bottom of a small stream.

Lyngbya martensiana Meneghini. Conspectus algologiae euganeae, p. 12. 1837. *Fig. 22.*

The plant mass was decidedly blue-green in color and composed of many filaments with thick and distinct sheaths, closely matted together. The cell measurements were 8.1–9.5 x 1.6–2.25 mic. In a few plants a thin calyptra was observed. Cross walls were marked by cyanophycin granules.

Durham, Durham County, N. C.

Lyngbya aestuarii (Mertens) Liebman. Bemerkninger og tilläg til den danske Algeflora. Kröyers Tidskrift, p. 492. 1841. (*Conferva aestuarii* Mertens. Jürg. alg. aquat. II, no. 8. 1816.) *Fig. 27.*

Two separate collections of this species were made, one in which the size of the trichomes fell just below the minimum size for the species, the other in which they were just under the maximum size. In the former, the plant mass was composed of comparatively few filaments, loosely arranged. The trichomes were provided with slightly tapering and capitate apices, with transverse walls marked by two rows of granules. In this collection, the cells measured 6.8–8.6 x 2–3.7 mic.

The plant mass in the second collection was of a much brighter blue-green color, and the filaments more compactly arranged. Here, too, the trichomes tapered at the ends and were capitate. Also the transverse walls were marked by two rows of granules. The cells measured 18.4–18.9 x 2.2–4.1 mic.

Hollow Rock, Durham County, N. C. Plants of both collections forming blue-green masses on moist earth near the water-level of a small creek.

MICROCOLEUS Desmazières, 1823

Plants living on soil, in fresh water or sometimes in salt water; filaments simple or vaguely branched, creeping on the ground, sometimes growing among other algae; sheaths colorless, more or less regularly cylindrical, not lamellose, in many species finally diffluent; trichomes many within the sheath in well developed filaments, closely crowded, often twisted into rope-like bundles; apex of trichome straight, tapering; apical cell acute, rarely obtuse conical, in one species capitate.

Microcoleus vaginatus (Vaucher) Gomont. Jour. de Bot. (Morot) **4:** 353. 1890; Monog. des Oscillariées, etc., p. 93, pl. 14, fig. 12. 1893. (*Oscillatoria vaginata* Vaucher. Histoire des Conferves d'eau douce, p. 200, pl. 15, fig. 13. 1803.) *Fig. 13.*

The plant mass was deep dark green in color. It was estimated that within a single filament there were about 80 trichomes. These had very distinct transverse walls, a few were capitate, and all were tapering at the apices. The cells varied in length from 3.1 to 4.1 mic. and displayed finely granular contents.

Durham, Durham County, N. C. Forming dark-colored irregular masses on wet soil in the greenhouse of Duke University.

SCHIZOTHRIX Kuetzing, 1843

Plants living on moist earth, dripping rocks, or in inundated places; filaments prostrate, caespitose, or forming erect or prostrate *Symploca*-like fascicles or a pannose stratum, frequently branched; sheaths thin or thick, colorless or variously colored, regular or irregular in outline, firm or diffluent, in some species encrusted with calcium carbonate and hardened; trichomes straight or spirally twisted, numerous, rarely solitary within the sheath.

a. Filaments agglutinated; trichomes evidently constricted at joints; cells 3–6 x 4–11 mic.................................... *S. friesii*
aa. Filaments not agglutinated; trichomes sometimes constricted at joints; cells 3.5 x 3.5–7 mic................................ *S. aikenensis*

Schizothrix friesii (Agardh) Gomont. Monog. des Oscillariées, etc., p. 54, pl. 9, fig. 1–2. 1893. (*Oscillaria friesii* Agardh. Synopsis algarum Scand., p. 107. 1817.) *Fig. 19.*

The plant mass, which was bright blue-green in color, consisted of a basal prostrate portion from which tufts of filaments arose to a height of 3 or 4 centimeters. The sheaths were somewhat roughened, containing sometimes one but usually two to four trichomes which were constricted at the joints and often moniliform. All of the sheaths were thick and colorless. The cells, with coarsely granular contents, measured 3.6–5.3 x 4.1–11.4 mic.

Hollow Rock, Durham County, N. C. Forming irregular, olive-green masses on damp soil and on a moist sandstone cliff.

Schizothrix aikenensis (Wolle) n. comb. (*Hypheothrix aikenensis* Wolle. Bull. Torrey Bot. Club **6**: 182. 1877.) *Fig. 24.*

The plant mass was of a dull blue-green color, very compact and thin. It consisted of densely matted filaments whose trichomes were sometimes constricted at the joints but never moniliform. Numerous granules were present in the cells. The cells measured 3.6 x 4.3–6.1 mic.

The species was originally described by Wolle as *Hypheothrix aikenensis* from a collection made at Aiken, S. C., by Ravenel. According to more recent systems of classification, the genus *Hypheothrix*, along with the genera *Symplocastrum*, *Chromosiphon*, and *Inactis*, is included as a section of the genus *Schizothrix*. This scheme is followed in the present report.

Durham, Durham County, N. C. Plant mass living on decaying vegetable matter in a stagnant pool on the Duke University campus.

Family PLECTONEMATACEAE Tilden, 1935

Filaments in free-floating caespitose masses or densely interwoven to form felts or mats; sheaths firm, rather thin, sometimes lamellose, hyaline or rarely yellow-brown; false branches frequent, usually in pairs; trichomes fitting closely within the sheaths, often slightly constricted between the cells; heterocysts absent; reproduction by hormogones.

PLECTONEMA Thuret, 1875

Plants growing among damp mosses, on wet rocks, or floating free; sheaths present; trichomes frequently constricted at the joints, exhibiting false lateral branching caused by rapid growth in certain portions, resulting in a rupture of the sheath; branches single or in pairs; apices straight, rarely tapering, without a calyptra.

Plectonema tenue G. Thuret. Ann. Sci. Nat. Bot., ser. 6, **1**: 380. 1875.

The more or less expanded plant mass was of a yellowish-brown color, due to the pigments in the sheaths. A sheath usually enclosed only a single trichome, but frequently two trichomes were observed. These gradually tapered at the apices. The cells measured 5.2–10.9 x 3.4–7.3 (rarely 8.1) mic. Terminating each trichome was a conical apical cell. The cell contents were either homogeneous or granular, and blue-green in color.

Marietta, Greenville County, S. C.

Family NOSTOCACEAE Naegeli, 1847

Colonies floating, single or gregarious, of indefinite, globose, ovoid or regularly lobate form or irregularly expanded, solid or hollow, soft or firmly gelatinous; sheaths more or less distinct, often not present, mostly confluent; trichomes unbranched, never tapering to a hair, the extremities usually similar; heterocysts present; reproduction by hormogones and hypnogonidia.

a. Heterocysts and hypnogonidia intercalary.
  b. Plant mass of indefinite shape........................*Anabaena*
  bb. Plant mass of definite shape..................................*Nostoc*
aa. Heterocysts terminal and hypnogonidia contiguous to them *Cylindrospermum*

ANABAENA Bory, 1822

Filaments, or trichomes, almost always aquatic, solitary, free-floating, or united loosely by the mucous teguments into a mucous stratum, never

approaching in consistency the tough thallus of *Nostoc;* sheaths usually lacking, in some species delicate or copious, gelatinous, hyaline, homogeneous, rarely confluent with the mucous layer; trichomes cylindrical or slightly tapering at the ends and with a conical apical cell, usually rigid and fragile, very rarely contorted, straight, spiral, or circinate, solitary or interwoven to form flocculent masses of small size; cells spherical to barrel-shaped, rarely cylindrical, never disk-shaped; heterocysts approximately spherical, always intercalary, numerous, solitary; reproduction by hormogones and hypnogonidia; hypnogonidia solitary or in short catenate series, spherical to cylindrical, generally much more elongated than those of *Nostoc*, either adjacent to the heterocysts or developed between them.

a. Plants living in the tissues of other plants................ *A. cycadeae*
aa. Plants not living in the tissues of other plants.
  b. Trichomes circinate; hypnogonidia cylindrical, curved.. *A. flos-aquae*
  bb. Trichomes straight or flexuous; hypnogonidia spherical, ovate or cylindrical, not curved.
    c. Hypnogonidia spherical, spherical-compressed, or ovoid *A. parva*
    cc. Hypnogonidia cylindrical.
      d. Hypnogonidia usually remote from the heterocysts, or contiguous to them on one side.................. *A. laxa*
      dd. Hypnogonidia always contiguous to and developing on both sides of the heterocysts.................. *A. torulosa*

Anabaena cycadeae J. Reinke. Bot. Zeit. 37: 473, pl. 6, fig. 1–5. 1879.

The habit of growth of this species apparently necessitates the formation of trichomes composed of only a few cells. The spherical to barrel-shaped cells varied considerably in size, measuring 2.7–6.3 x 2.7–7.2 mic. Most of the heterocysts were spherical, measuring 5.4–6.3 x 5.4–6.3 mic. in size.

Durham, Durham County, North Carolina. Causing the formation of long, branched nodules on the roots of *Zamia florida* in the greenhouse of Duke University.

Anabaena flos-aquae (?) (Lyngbye) Brebisson. In Brebisson and Codey, Algae des environs de Falaise, p. 36. 1835. (*Nostoc flos-aquae* Lyngbye. Tentamen hydrophytologiae danicae, p. 201, table 68. 1819.)

The absence of gonidia in the materials studied makes this identification uncertain. The presence of pseudovacuoles indicates the plant to be a water-bloom species. Because all available measurements agree

with the corresponding descriptions for this species, the writer places the few scattered plants that were observed in this species. The spherical compressed vegetative cells measured 4.5–5.9 mic. in diameter, and the heterocysts were slightly larger.

Durham, Durham County, N. C. Intermixed with other algae growing in a small creek in the Duke Forest.

Anabaena parva sp. nov. *Fig. 25.*

Plantae massa glutinosa, expansa in solo humido aut libere fluitante, caerulei-viridi; vaginis invisibilibus; trichomatibus modice rectis, constrictis ad articulos; cellis ovatis vel ellipticis in forma; heterocystis sphaericis vel ellipticis; gonidiis sphaericis vel ellipticis in forma, numerosis in serie catenata, remotis ab heterocystis, evolutis centrifuge, continentibus multa et magna granula cyanophycina; contento cellae paris generis, caerulei-viridi.

Cellis 2.9–4.1 x 4.1–8.2 mic.
Heterocystis 4.1–5.3 x 4.5–8.2 mic.
Gonidiis 4.5–7.4 x 3.6–8.2 mic.

Plant mass gelatinous, expanded, spreading on damp soil or free-floating, blue-green; sheaths invisible; trichomes moderately straight, constricted at the joints; cells oval or elliptical in shape; heterocysts spherical or elliptical; gonidia spherical or elliptical in shape, numerous in catenate series, remote from the heterocysts, developing centrifugally, containing numerous large cyanophycin granules; cell contents homogeneous, blue-green.

Cells 2.9–4.1 x 4.1–8.2 mic.
Heterocysts 4.1–5.3 x 4.5–8.2 mic.
Gonidia 4.5–7.4 x 3.6–8.2 mic.

Anabaena variabilis Kuetzing is the species most closely indicated by the appearance of the plants of this collection. There can be no confusion, however, after careful study. The plants of this new species are much smaller in every respect.

Myrtle Beach, Horry County, S. C. Forming large, irregular gelatinous masses floating on the surface of Lake Chapin. Collection:—S. C. 42. July 4, 1933.

Anabaena laxa (Rabenh.) A. Braun. Bull. Soc. Bot. Fr. **32**: 120, pl. 4, fig. 2–3. 1885. (*Sphaerozyga laxa* Rabenh. Fl. eur. algarum **2**: 193. 1865.) *Fig. 34.*

The trichomes of this collection were quite straight. Heterocysts, which were usually elliptical in shape, measured 4.5–6.3 x 6.1–9 mic.

The spherical or short-cylindrical cells were 4.1–4.5 x 3.2–4.1 mic. The distinguishing feature of this species, and one that separates it from *Anabaena inequalis* (Kuetz.) Bornet and Flahault, is the colorless wall of the gonidium. The hypnogonidia were cylindrical and measured 5.4–6.3 x 12.6–20.5 mic.

Old Fort, McDowell County, N. C. Forming a thin, blue-green coating on mud in the bottom of a small creek.

Anabaena torulosa (Carmichael) Lagerh. Oefversigt af Kengl. Vetensk. Akad. Förhandl. **40**: 47. 1883. (*Belonia torulosa* Carmichael. In Hooker, British Flora **2**: 379. 1833.) *Fig. 33.*

The vegetative cells that were measured for this determination fell within the limits of 3.9–4.8 x 2.6–3.6 mic. and were spherical or barrel-shaped. All of the heterocysts were nearly spherical and measured 5.4–7.2 mic. in diameter. Adjacent to the heterocysts were long, cylindrical gonidia, 7.2–9 x 10.8–19.8 mic. They developed centripetally.

Hollow Rock, Durham County, N. C. Forming gelatinous masses near the edge of a small stream.

NOSTOC Vaucher, 1803

Colonies solid or hollow, mucous, gelatinous, or coriaceous, composed of a more or less firm jelly with a denser limiting layer, usually globose or oblong when young, later either remaining so or assuming a variety of forms in the various species, frequently breaking open to form extensive flattened expansions, often with lacerated margins, terrestrial or aquatic, attached to a substratum or free-floating; filaments flexuous, densely intertwined and contorted, generally more closely crowded toward the exterior; sheaths often not present or inconspicuous, sometimes distinct in portions of the colony, especially near the periphery, colorless or not uncommonly yellow or brown, firm, thick, mucous or gelatinous, mostly confluent, the gelatinous colony being made up of dissolved individual sheaths; trichomes torulose or moniliform, consisting of a single row of uniform cells, unbranched, showing no differentiation of base and apex; vegetative cells spherical, barrel-shaped or cylindrical; heterocysts larger than, but generally the same shape as, the vegetative cells, spherical to oblong, terminal or intercalary, often in series; reproduction by means of hormogones and hypnogonidia; gonidia spherical to oblong, intercalary, seriate, beginning midway between the heterocysts and developing centrifugally.

a. Colonies microscopic.
   b. Filaments loosely flexuous; sheaths wide, bulbous; trichomes distinct *N. paludosus*
   bb. Filaments densely entangled; sheaths close, transparent; trichomes scarcely distinct................................*N. punctiforme*

aa. Colonies not microscopic.
   b. Cells 2.3–3.6 x 10.8–19.8 mic., cylindrical; hypnogonidia oval, elliptical, or cylindrical, frequently curved *N. ellipsosporum* var. *minimum*
   bb. Cells 3–4 x 7–8.6 mic., barrel-shaped or cylindrical; hypnogonidia oblong, never curved .. ..*N. muscorum*

Nostoc paludosum Kuetz. Tab. phycol. **2:** 1, pl. 1, fig. 2. 1850.

The colonies, somewhat microscopic, are larger than those of *Nostoc punctiforme* (Kuetzing) Hariot. The trichomes were provided with very evident inflated sheaths. Measurements of the cells fell within the limits of 3.2–3.6 x 3.2–5.6 mic. The spherical-shaped heterocysts measured 4.2 mic. in diameter. Gonidia were not observed. Granules were abundant within the cells.

Hollow Rock, Durham County, N. C. Embedded in a gelatinous mass formed by other algae. On the wet bank of a small stream.

Nostoc punctiforme (Kuetz.) Hariot. Jour. de Bot. (Morot) **5:** 30. 1891. (*Polycoccus punctiforme* Kuetz. Phycol. generalis, p. 171. 1843.) *Fig. 23.*

The colonies, always microscopic in size, were spherical or somewhat flattened in shape, and contained, usually, only a few much twisted and contorted trichomes. In form, the cells were quite variable, being, usually spherical or spherical-compressed. They measured 3.2–4 x 3.2–4 mic. The heterocysts were few in number, and small. No gonidia were observed.

Hollow Rock, near Durham, Durham County, North Carolina. Forming, with other unicellular species of blue-green algae, a thin coating on submerged rock in a nearby stream.

Nostoc ellipsosporum (Desmaz.) Rabenh. var. **minimum** var. nov. *Fig. 21*.

The plants under consideration differ from *Nostoc ellipsosporum* (Desmaz.) Rabenhorst only in the size of the vegetative cells, which are much smaller and longer than in the species. In the species the cells measure 4 x 6–14 mic., while in this new variety they measure 2.4–3.6 x 10.8–24 mic. The heterocysts and gonidia conform to those of the

variety both in size and shape, with the exception that the former appear to be slightly broader in the species.

Alligator Lake, near Myrtle Beach, Horry County, South Carolina. Forming blue-green or brown gelatinous masses among mosses in damp places on the bank of the lake.

Nostoc muscorum (?) Agardh. Dispositio algarum sueciae, p. 44. 1812.

Gonidia were not present in this collection and thus the writer cannot be certain of this identification. He is, however, reasonably satisfied. The much twisted and contorted trichomes measured 3.5–4.5 mic. in diameter, and were composed of barrel-shaped cells 7–8.6 mic. in length. In size, the globose heterocysts varied between the limits 5.2–6.8 mic. in diameter. Cell contents were quite granular.

Durham, Durham County, North Carolina. Forming expanded gelatinous masses on moist soil and among mosses. Collected in the Duke Forest.

CYLINDROSPERMUM Kuetzing, 1843

Sheaths diffluent, forming an amorphous, expanded mucous stratum of indefinite form; trichomes relatively short because of frequent hormogone formation, sometimes exhibiting movement; vegetative cells cylindrical, longer than broad; heterocysts cylindrical, longer than broad, solitary, always terminal; hypnogonidia always developed from the cell or cells next to the heterocyst at base of plant, generally solitary, rarely in series, outer layer of wall papillate in some species.

a. Wall of gonidium smooth.
  b. Hypnogonidia 9-21 x 20-30 mic., with radial striations in outer wall. . . . . *C. alatosporum*
  bb. Hypnogonidia 9-12 x 10-20 mic., without such striations *C. muscicola*
aa. Wall of gonidium covered with numerous short needle-like spines *C. trichotospermum*

Cylindrospermum alatosporum F. E. Fritsch. Ann. of the South African Mus. 9: 578, fig. 37, d-h. 1911-1918. *Fig. 20.*

Vegetative cells studied from this collection measured 3.6 x 3.6–4.8 mic.; heterocysts were 4.5–5.1 x 6.2–7.5 mic.; hypnogonidia were 7–12 x 16.6–22 mic. It will be noticed that a few of these measurements are a little smaller than those of the typical species, but not to such an extent as to affect the ultimate identification. Most of the vegetative cells

were barrel-shaped. The ellipsoid gonidia developed singly and possessed rounded instead of truncate apices. The peculiar and characteristic striations of the walls of the gonidia were well in evidence.

Durham, Durham County, N. C. Associated with *Vaucheria* sp. in freshwater in a laboratory at Duke University.

Cylindrospermum muscicola Kuetz. Phycol. germanica, p. 173. 1845. *Fig. 18.*

The slimy plant mass was dark green in color. Vegetative cells measured 3.2–3.6 x 2.5–4.1 mic.; the heterocysts 5.4–6.3 x 5.4–7.2 mic.; the gonidia 8.1–8.2 x 16.4–19.8 mic. The cells were somewhat quadrate in shape and constricted at the joints. The measurements of the heterocysts were slightly larger than those for the species, but this was not of sufficient importance to alter the identification.

Hollow Rock, Durham County, N. C. Forming a thin coating on moist clay on the bank of a small stream.

Cylindrospermum trichotospermum Frémy. Les Myxophycées de l'Afrique équatoriale francaise, p. 379, fig. 316. 1930. *Fig. 26.*

This species is characterized by the elliptical gonidia, the walls of which are provided with numerous needle-like spines. The vegetative cells from this collection measured 3.2–3.6 x 4.5–7.2 mic.; the heterocysts 4.5–5.5 x 8.1–9 mic.; the gonidia 11.8–14.1 x 19.8–28.8 mic.

In the original description of this species the plant mass is said to be "minute, floating among other algae." The North Carolina material, however, occurred on mud forming plant masses about the size of a silver dollar. These masses were composed of only a single species.

Old Fort, McDowell County, N. C. Plant masses, formed on mud, shaded by tall sedges.

Family SCYTONEMATACEAE (Kuetzing) Rabenhorst, 1865

Filaments differentiated into a basal and terminal portion, simple or branched; false branches formed by the perforation of the sheath by the trichome, which thereupon issues as one or two long flexuous branches, each developing a sheath of its own; sheaths homogeneous and colorless, or lamellose and yellowish or brownish, firm and tubular; trichomes consisting of a single row of cells, one or more included within the sheath; heterocysts and hypnogonidia variously disposed; reproduction by means of hormogones and hypnogonidia.

a. False branches usually arising between two heterocysts, single or in pairs; sheaths delicate or very thick, homogeneous or lamellose; lamellae parallel, or more or less diverging toward the apex .... *Scytonema*

aa. False branches usually arising in the immediate region of the heterocysts, single; sheaths somewhat thin, flexuous, more or less fragile .. *Tolypothrix*

SCYTONEMA C. A. Agardh, 1824

Plants generally forming a dense interwoven mass on damp or wet substrata, more rarely submerged; filaments branched, containing a single trichome; sheaths tubular, of practically uniform thickness, delicate or thick, firm, homogeneous and colorless, or lamellose, with parallel or more or less diverging layers, their edges in the latter case sometimes projecting and producing a frayed margin, termed ocrea, yellowish or brown in color; false branches long and flexuous, developing a sheath of their own, arising singly or more commonly in pairs between two heterocysts, formed by the lateral perforation of the sheath by the trichomes at first pushing out as a loop, which later breaks across the apex; trichomes straight, cylindrical, toward the growing end of the filament, often increasing in diameter, the cells becoming shorter and rounded; heterocysts intercalary; reproduction normally by hormogones, and by hypnogonidia in many species; hormogones formed at the ends of branches; hypnogonidia spherical or oval, with a thin, smooth wall.

a. Sheaths homogeneous or lamellose, not with diverging layers.
  b. Plants aquatic .... *S. crispum*
  bb. Plants aerial or subaerial.
    c. Sheaths homogeneous; trichomes increasing in diameter toward the apices; cells 5–12.8 mic. in diameter .... *S. carolinianum*
    cc. Sheaths lamellose; trichomes more or less constant in diameter throughout; cells 25–33 mic. in diameter .... *S. insigne*
aa. Sheaths lamellose, with diverging layers.................. *S. crustaceum*

Scytonema crispum (Agardh) Bornet. Bull. Soc. Bot. Fr. **36:** 156. 1889. (*Oscillatoria crispum* Agardh. Synopsis algarum Scand., p. 108. 1817.)

The plant mass was woolly in appearance and greenish blue-green in color. All of the filaments measured between 18 and 31.5 mic. in diameter, with sheaths sometimes lamellose. False branches were always in pairs, and most of the trichomes were constricted at the joints. The numerous heterocysts were slightly broader than the vegetative cells and depressed-spherical to quadrate in shape. The cells measured 15–29 x 5–10.5 mic. in size, with slightly granular contents and a bright blue-green color.

Lake Chapin, near Myrtle Beach, Horry County, S. C. Forming large woolly masses attached to *Chara* sp. and other plants growing in the lake.

Scytonema carolinianum sp. nov. *Fig. 15–16.*

Filamentis cum aliis algis mixtis, assequentibus diametrum maximum ad apices et decrescentibus ad mediam regionem; falsis ramis longis, plerumque in paribus, agglutinatis aliquod spatium a base; vaginis sine colore, latis, mucosis, homogeneosis; trichomatibus 5 mic. in diametro prope mediam regionem, crescentibus ad 12.8 mic. ad apices; minima longitudine cellarum attacta in meristema parte ad fines trichomatum; heterocystis sphaericis, vel brevi-cylindratis; contento cellarum granulato, viridi usque ad caerulei-viridem in colore.

Filamentis 10.8–28.5 mic. in diametro,
Cellis 5–12.8 x 2.4–14.8 mic.
Heterocystis 8.3–10.5 x 8.3–12.2 mic.

Filaments intermixed with other algae, reaching their maximum diameter at the apices and decreasing toward the mid-region; false branches long, usually in pairs, agglutinated for some distance from the base; sheaths colorless, wide, mucous, homogeneous; trichomes 5 mic. in diameter near the mid-region, increasing to 12.8 mic. at their apices; minimum length of cells reached in the meristem portion at the ends of the trichomes; heterocysts spherical, or short cylindrical; cell contents granular, green to blue-green in color.

Filaments 10.8–28.5 mic. in diameter.
Cells 5–12.8 x 2.4–14.8 mic.
Heterocysts 8.3–10.5 x 8.3–12.2 mic.

This species appears to be characterized by the gradual diminution in diameter of the trichomes from the apices to the mid-region. The plants were abundant in a single collection.

Hollow Rock, Durham County, N. C. Filaments intermixed with those of *Stigonema hormoides* (Kuetz.) Bornet and Flahault. Growing on damp soil rich in decaying vegetable matter. Collection:—5a.

Scytonema insigne W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35: 266.** 1897.

This plant proved to be very interesting, not having been collected since W. and G. S. West first described it from Africa in 1897. The plant mass, greenish in color, was somewhat widely expanded. The filaments, measuring 18–26.5 mic. in diameter, formed erect tufts.

Each filament was provided with a thick, lamellose sheath formed of parallel layers. The yellowish color of the inner layers of the sheath, observed here and there, was accentuated by the colorless outer ones. The cells measured 8.2–12.3 x 3.6–16.4 mic. and appeared constricted at the joints.

Hollow Rock, Durham County, N. C. Growing in patches on wet soil and on damp limestone rocks.

Scytonema crustaceum Agardh. Systema algarum, p. 39. 1824. *Fig. 17.*

The plant mass was brown in color, widely expanded, and calcareous but somewhat velvety to the touch. It consisted of filaments, 16–28.8 mic. in diameter, whose false branches occurred in pairs at the base of the mass, but only singly in the erect branches. All of the sheaths were firm and thick, with decidedly diverging layers, and very irregular in outline. The cells were somewhat spherical-depressed in shape, usually shorter than broad, and constricted at the joints. Most of the heterocysts were spherical to spherical-compressed. Cell contents appeared to be either homogeneous or with a few large granules.

Myrtle Beach, Horry County, S. C. Forming widely expanded, but relatively thin, brown patches on dry ground near Lake Chapin.

TOLYPOTHRIX Kuetzing, 1843

Plants chiefly aquatic, occurring among various plants in ponds and lakes, forming floccose floating masses or submerged tufts, or, rarely, a stratum on damp rocks; filaments branched; sheaths rather thinner than those of *Scytonema*, colorless or yellowish or brown, either flexible or more or less fragile; false branches more or less frequent, usually single and mostly issuing from the main filament in the immediate region of the heterocysts, very rarely between two heterocysts; heterocysts single or from 2 to 5 in a series; reproduction normally by means of hormogones, sometimes by hypnogonidia; hormogones formed at the ends of the branches; hypnogonidia spherical, oval or ellipsoid, often many in a series, with smooth, thin walls.

a. Filaments under 5 mic. in diameter........................*T. delicatula*
aa. Filaments over 5 mic. in diameter.
 b. Plants aquatic..*T. distorta*
 bb. Plants aerial, living on trunks of trees................*T. rechingeri*

Tolypothrix delicatula sp. nov. *Fig. 28.*

Plantae massa lanata, spinulari forma, colore caerulei-viridi, filamentis rare scissis, ramis falsis brevibus et patulis; vaginis maxime

astrictis, indistinctis nisi ad fines trichomatum, sine colore; cellis non omnino vel vix constrictis ad articulos; heterocystis, prope ramos, sphaericis usque ad oblongos, qui sunt remoti a ramis cadiformibus usque ad cylindratos; partibus interioribus caerulei-viridibus, habentibus granula sparsa.

Filamentis 4.1–4.5 mic. in diametro.

Cellis 4.1–4.5 x 12.3–18.4 mic.

Heterocystis 4.1–6.9 x 5.3–31.5 mic.

Plant mass woolly, cushion-shaped, blue-green in color; filaments rarely branched; false branches short, spreading; sheaths very close, indistinguishable except at the ends of the trichomes, colorless; cells not at all or scarcely constricted at the joints; heterocysts, adjacent to the branches, spherical to oblong, those remote from the branches barrel-shaped to cylindrical; cell contents blue-green, containing scattered granules.

Filaments 4.1–4.5 mic. in diameter.

Cells 4.1–4.5 x 12.3–18.4 mic.

Heterocysts 4.1–6.9 x 5.3–31.5 mic.

This material was collected in abundance and studied in both the living and in preserved conditions. At first glance, the appearance of the plant is similar to that of *Tolypothrix tenuis* Kuetzing, but it differs from this species in several characteristics. In the plants described above the sheath is so thin that it is invisible except at the ends of the trichomes, while in *T. tenuis* the sheath is seen distinctly in any portion of the plant.

The cells of *T. tenuis* rarely fall below 5 mic. in diameter and they are as long as broad or sometimes longer than broad. The heterocysts are spherical. In the case of the plants of the new species, on the other hand, the cells never exceed 4.5 mic. in diameter and are 12.3 to 18.3 mic. in length, making them cylindrical in shape. The heterocysts are spherical, barrel-shaped, or cylindrical.

Alligator Lake, Horry County, S. C. Forming slimy, irregularly expanded, submerged masses on an unidentified species of *Chara*. Collection:—S. C. 3. July 2, 1932.

Tolypothrix distorta (Flora Danica) Kuetz. Phycol. generalis, p. 228. 1843. Flora Danica, pl. 820. 1780.

The plant mass was more or less expanded, blue-green in color, and consisted of filaments which varied in diameter from 11.2 to 14.1 mic. False branches were very abundant, and long and graceful in appearance. The trichomes, which possessed thin and colorless sheaths, were 8.2 to 10.1 mic. in diameter, with a few below the minimum for the

species. Cells of this species, many of which were constricted at the joints, measured 2.5 to 9.4 mic. in length. Their contents appeared homogeneous and blue-green in color.

Greenville, Greenville County, S. C. Forming a more or less expanded, blue-green coating on the bottom of a shallow pool.

Tolypothrix rechingeri (Wille) Geitler. In Pascher, Die Süsswasser-Flora Deutschlands, etc., **12:** 259, text fig. 309. 1925. (*Hassallia rechingeri* Wille. Süsswasseralgen von den Samoainseln, etc. Botanische und Zoologische ergebnisse einer wissenschaftlichen forschungsreise nach den Samoainseln von März bis Dezember 1905, p. 10, pl. 1, fig. 19–26. 1914.) *Fig. 31.*

The plants collected agree in every way with the species description except that some of the trichomes were as small as 6.1 mic. in diameter. Wille in his original description states that the trichomes branch between the heterocysts, but this does not seem always to be the case. The plant mass was brown in color. The filaments measured 11 to 15 mic. in diameter, were richly branched, and were provided with yellow, very fragile sheaths. Usually, the heterocysts were spherical in shape, sometimes spherical-depressed.

Durham, Durham County, N. C. Growing in loose masses on the trunk of a cedar tree near Duke University.

Family STIGONEMATACEAE Kirchner, 1898

Plants (filaments) free, or forming a stratum or gelatinous colony, composed of a single series of cells, or of two or more series, within a sheath, resulting from cell division in one, two, or three planes, branched; true branches present; cells somewhat spherical, cylindrical, or irregularly angular; heterocysts intercalary or terminal on short lateral branches; reproduction by hormogones and by hypnogonidia.

a. Trichomes consisting of a single row of cells; branches usually unilateral *Hapalosiphon*

aa. Trichomes consisting of one to several rows of cells; branches scattered, usually not unilateral . . . . *Stigonema*

HAPALOSIPHON Naegeli, 1849

Plant mass caespitose-floccose, thin, usually aquatic; filaments free, in floating patches or epiphytic on higher plants, branched, consisting typically of a single row of cells (rarely of two rows) within the sheath; branches erect, usually about the same diameter as the creeping primary

filament, commonly unilateral, long, flexuous, very slightly tapering; sheaths firm, narrow, of uniform thickness; hypnogonidia with thick, yellowish-brown walls, often formed in abundance.

a. Filaments 5.5–7.5 mic. in diameter; branches short, equal to, sometimes slightly thinner than, the main filament.............. *H. welwitschii*
aa. Filaments 7.2–9.5 mic. in diameter; branches long, flexuous, always thinner than the main filament.............................. *H. hibernicus*

Hapalosiphon welwitschii W. and G. S. West. Welwitsch's African freshwater algae. Jour. Bot., Brit. and Foreign **35**: 242. 1897. *Fig. 29.*

The main filaments observed measured 6.4 to 6.6 mic. in diameter. They occurred singly. Numerous unilateral branches were present, all of which were slightly smaller than those of the parent filaments. The cells were quite variable in size, those in the branches being longer than those in the main filament.

Hollow Rock, Durham County, N. C. Intermixed with an unidentified species of *Schizothrix*, growing near the edge of a small creek.

Hapalosiphon hibernicus (?) W. and G. S. West. Jour. Royal Micros. Soc., 1896, p. 163.

Sufficient material was not at hand to insure a positive identification. The filaments occurred singly, and measured 8.2–9.4 mic. in diameter. Cells within the main filaments fell within the limits 5.3–7.1 x 4.1–10.2 mic. The long cylindrical heterocysts were 5.6–6.2 x 18.4–20.5 mic. in size. Because these measurements are fairly suitable for the corresponding measurements for the species, the writer, with hesitancy, places the few plants in the collection in this species.

STIGONEMA C. A. Agardh, 1824

Plants, or filaments, free, terrestrial or aquatic, free-floating or aggregated to form rigid or soft felt-like or cushion-like dark brown masses on damp surfaces, frequently very wide, irregular, and often richly provided with short, thick, irregularly disposed branches that are often considerably narrower than the main filaments; sheaths extremely thick, lamellose, generally golden-yellow or brown, made up of dissolved sheaths of the contained cells, which as they grow continue to excrete gelatin; trichomes consisting of rounded cells exhibiting obvious protoplasmic connections, usually with distinct individual sheaths disposed in one, two, or three to several rows; heterocysts commonly lateral,

here and there intercalary; reproduction normally by hormogones developed in the ends of ordinary branches or in short special branches, few- or many-celled.

a. Trichomes consisting of a single series of cells.
  b. Filaments 7–15 mic. in diameter; sheaths usually colorless ... *S. hormoides*
  bb. Filaments 24–26 mic. in diameter; sheaths thick, lamellose, yellowish or brownish........ *S. panniforme*
aa. Trichomes consisting of two to several series of cells.
  b. Filaments 27–37 mic. in diameter; hormogones 12 x 45 mic. in size ... *S. turfaceum*
  bb. Filaments 40–70 mic. in diameter; hormogones 18 x 45 mic. in size ... *S. informe*

Stigonema hormoides (Kuetz.) Bornet and Flahault. Ann. Sci. Nat. Bot., ser. 7, **5**: 68. 1887. (*Scytonema hormoides* Kuetz. Phycol. generalis, p. 215. 1843.) *Fig. 32.*

Three features characterize this species: the comparatively small size of the filament, the colorless sheath, and the uniseriate axis. The filaments studied from this collection measured 8–12 mic. in diameter. Most of the vegetative cells were spherical and were 4.1–4.9 mic. in diameter.

Hollow Rock, Durham County, N. C. Growing in loose mats on damp soil well shaded by forest trees.

Stigonema panniforme (C. A. Agardh) Kirchner. Kryptogamen-Flora von Schlesien **2**: 230. 1878. (*Scytonema panniforme* Agardh. Synop. algarum, p. 116. 1817.)

This species is characterized by the definite size of the filaments, which contain each a single row of cells, and by the thick, yellow lamellose sheaths. The filaments in this collection measured 24.6–28.7 mic. in diameter. The hormogones were 102.5 mic. in length and contained 15 to 22 cells each. Most of the branches tapered toward the apices. The cells, frequently discoid in shape, measured 8.2–12.3 x 4.1–14.5 mic.

Hollow Rock, Durham County, N. C. Growing on moist soil near a small creek and on the surface of decaying vegetable matter.

Stigonema turfaceum (Berk.) Cooke. British freshwater algae, p. 273. 1884. (*Scytonema turfaceum* Berk. English Botany, pl. 2826, fig. 1. 1838.) *Fig. 44.*

Filaments from this collection measured 28.7–36.9 mic. in diameter. This species is terrestrial, the trichomes of which are composed of several rows of cells. The latter measured 5–12.5 x 3.5–13 mic. Hormogones were quite variable in size, some of them falling short for the minimum of the species. Each consisted of from 7 to 13 cells.

Hollow Rock, Durham County, N. C. Growing on moist earth in rocky places, associated with *Stigonema hormoides.*

Stigonema informe Kuetz. Species algarum, p. 319. 1849.

When collected, the widely expanded plant mass was dark brown in color. All of the filaments were large, coarse, and very irregular in outline, each containing several rows of cells. The filaments were 32.8–59.3 mic., the cells 11.2–17.2 mic. in diameter. Hormogones were numerous, measuring 30 to 50 mic. in length.

Hollow Rock, Durham County, N. C. Forming expanded masses on damp, well shaded soil near the bank of a small stream.

Family RIVULARIACEAE Rabenhorst, 1865

Filaments cohering by their gelatinous sheaths, forming more or less definite spherical or hemispherical colonies, radiating from the center outward, or parallel and forming caespitose strata, rarely solitary, simple or branched; trichomes tapering from base to apex, ending in a colorless hair, one to several within a sheath; branches false, usually beneath the heterocysts, more rarely between the heterocysts; heterocysts basal or intercalary, rarely absent; reproduction by means of hormogones and hypnogonidia.

a. Filaments not agglutinated, rarely branched; hypnogonidia usually not present
Calothrix

aa. Filaments agglutinated, widely branched; hypnogonidia present
Gloeotrichia

CALOTHRIX C. A. Agardh, 1824

Plants sometimes solitary, more usually forming penicillate tufts or soft velvety expansions, which are generally attached to submerged rocks and stones, some species being epiphytic; filaments simple or with scanty false branches; sheaths cylindrical, firm, frequently thick and lamellose, often diffluent in the upper portion, sometimes brown in color; trichomes tapering from near the base upward, or only at the upper end, sometimes long and flexuous, terminating in a point or fre-

quently in a long hyaline hair; heterocysts basal or intercalary, single or several in a series, absent in a few species; hormogones often formed in series; hypnogonidia formed singly or in a series adjacent to the basal heterocyst.

a. Filaments epiphytic, isolated or in small groups............*C. genuflexa*
aa. Filaments not epiphytic, forming more or less expanded masses, sometimes free-floating; sheaths colorless........................*C. braunii*

Calothrix genuflexa sp. nov. *Fig. 30.*

Filamentis usque ad 350 mic. in longitudine, seiunctis vel parvis globis, epiphyticis; parte infirma filamenti flecta et hospiti affixa, apice libera et recta; vaginis densis, firmis, sine colore, tubulata; trichomatibus constrictis ad articulos, valde paulatim fastigalis, non in pilum finitis; cellis cadiformibus, brevioribus in regione basis; heterocystis singulis, basalibus, hemisphaericis, sine colore; rebus interioribus cellarum paululum granulatis, caeruli-viridibus.
Filamentis 9.6–12.8 mic. in diametro.
Cellis 8.2–12.7 x 7.2–11.5 mic.
Heterocystis 6.4–11.5 mic. in diametro.

Filaments up to 350 mic. in length, isolated or in small groups, epiphytic; basal portion of filament bent, attached to the host, apical portion free and erect; sheaths thick, firm, colorless, tubular; trichomes constricted at the joints, very gradually tapering, not ending in a hair; cells barrel-shaped, shorter in the basal region; heterocysts single, basal, hemispherical, colorless; cell contents somewhat granular, blue-green.
Filaments 9.6–12.8 mic. in diameter.
Cells 8.2–12.7 x 7.2–11.5 mic.
Heterocysts 6.4–11.5 mic. in diameter.

The new plant compares unfavorably with any species of *Calothrix* yet described. *Calothrix epiphytica* West and West and *C. scytonemicola* Tilden make the nearest approach. When compared with the former, the lengths of the filaments, their habit of growth, and the position of the heterocysts are identical with those of the new species. On the other hand, the diameters of the trichomes and the filaments of the proposed new species are twice those of *C. epiphytica*, and the trichome does not end in a hair, as is the case with the latter.

The plants differ from those of *C. scytonemicola* in having a much broader filament, and heterocysts not in pairs. The sheath is firm and thick, while in *C. scytonemicola* it is indistinct.

Florence, Florence County, S. C. Epiphytic on filaments of *Scytonema crispum* Ag. that were attached to a log floating in Muldrow's Mill Pond. Collection:—S. C. 31. July 3, 1933.

Calothrix (?) **braunii** Bornet and Flahault. Ann. Sci. Nat. Bot., ser. 7, **3**: 368. 1886.

The free-floating filaments were not present in sufficient numbers to allow a satisfactory identification. They measured 6–10.3 mic. in diameter, the smaller sizes referring to young filaments. The sheaths were thin, colorless, and close, visible only near the base of the plants. The trichomes measured 5.6–7.2 mic. in diameter. They tapered gradually from the base and ended in long hairs. The cell contents were homogeneous and blue-green.

Hollow Rock, Durham County, N. C. Free-floating and attached to dead twigs in a creek.

var. **maxima** var. nov. *Fig. 35.*

Except in size, the plants agree in every way with *Calothrix braunii*. With the plants under consideration, however, the diameter of the filaments may be greater by 4 mic., and that of the trichome by 2–3 mic. Also the sheath is occasionally yellowish, which is never the case in *C. braunii.*

Gaffney, Cherokee County, S. C. Forming a thin coating on the concrete bottom and sides of an outdoor lily pool located in the yard of the author's home.

GLOEOTRICHIA J. G. Agardh, 1842

Colonies macroscopic, at first attached, later often free-floating, solid when young, becoming inflated and hollow, soft, spherical or nearly so; filaments radiating from the center outward, repeatedly branched, false branches obvious only in very young colonies; sheaths conspicuous only near the base of the trichomes, being gelatinous and confluent in outer parts of the colony, sometimes yellow or brown, in some cases indurated with lime when mature; trichomes more or less torulose, tapering from base to apex; heterocysts usually basal, intercalary at points of branching; hormogones formed from upper parts of the trichomes, the apical hair being shed; hypnogonidia cylindrical, elongated, developed from the cell immediately above the heterocyst and remaining for some time within the basal part of the protecting sheath.

Gloeotrichia echinulata (J. E. Smith) Richter. Forschungsbr. Biol. Stat. zu Plön **2**: 31, fig. 1–8. 1894. (*Conferva echinulata* Smith. English Botany, pl. 1378. 1804.)

Free-floating filaments of these plants were collected in great abundance, they being no longer held together in colonies. The trichomes

measured 8–10 mic. in diameter at the base, tapering gradually from base to apex and ending in a long hair. Sheaths were distinct in the basal regions of the trichomes. The spherical heterocysts were terminal and measured 8–12.5 mic. in diameter. Granules were abundant within the cells.

Alligator Lake, Horry County, S. C. Not in distinct colonies, but forming slimy, mucilaginous masses attached to a species of *Chara*.

DEPARTMENT OF BIOLOGY,
UNIVERSITY OF SOUTH CAROLINIA,
COLUMBIA, S. C.

LITERATURE LIST

(A selected list representing about one-third the original bibliography)

AGARDH, C. A. Dispositio algarum sueciae, pp. 35–36, 44. Lund. 1812.

——— Algarum decades **2**: 25. Lund. 1813.

BORNET, E., AND C. FLAHAULT. Revision des Nostocacées hétérocystées contenues dans les principaux herbiers de France. Ann. Sci. Nat. Bot., ser 7, **3**: 323–381; **4**: 343–373; **5**: 51–129; **7**: 177–262. 1886–1888.

COOKE, M. C. British freshwater algae **1**: 1–329; **2**: pl. 1–130. 1884.

FORTI, A. Myxophyceae. In De Toni, J. B., Sylloge algarum **5**: 1–761. 1907.

FRÉMY, P. Les Myxophycées de l'Afrique équatoriale française. Arch. Bot. **3**, Mém. **2**: 1–507, fig. 1–362. 1930.

GEITLER, L. Cyanophyceae. In Pascher, A., Die Süsswasser-Flora Deutschlands, Oesterreichs und der Schweiz, Heft **12**: 1–450, text fig. 1–560. 1925.

——— Cyanophyceae. In Dr. L. Rabenhorst's Kryptgamen-Flora von Deutschland, Oesterreich und der Schweiz **14**: 1–1195, text fig. 1–780. 1931–1932.

GOMONT, M. Essai de classification des Nostocacées homocystées. Jour. de Bot. **4**: 349–357. 1890.

——— Monographie des Oscillariées (Nostocacées homocystées). Ann. Sci. Nat. Bot., ser. 7, **15**: 263–368, pl. 6–14; and (2nd part) **16**: 91–264, pl. 1–7. 1892.

KEUTZING, F. T. Phycologia generalis, pp. 171, 185, 215, 228. Leipzig. 1843.

——— Tabulae phycologicae **1**: 1–54, pl. 1–100. 1845–49.

——— Species algarum. Leipzig. 1849.

RABENHORST, L. Flora europaea algarum **2**: 50, 63, 64, 102. Leipzig. 1865.

SMITH, G. M. Phytoplankton of the inland lakes of Wisconsin. Bull. Wisconsin Geol. and Nat. Hist. Surv. **57**: 1–227, pl. 52–88. 1920.

TILDEN, J. E. Minnesota algae **1**: 1–328, pl. 1–20. 1910.

——— Standardization of method for drawing algae for publication. Bot. Gaz. **95**: 515–518, fig. 1–19. 1934.

THURET, G. Essai de classification des Nostochinées. Ann. Sci. Nat. Bot., ser. 6, **1**: 372–382. 1875.

WOOD, H. C. Prodromus of a study of the freshwater algae of eastern North America. Proc. Amer. Phil. Soc. **11**: 119–145. (1869) 1871.

WOOD, H. C. A contribution to the history of the fresh-water algae of North America. Smithsonian Contrib. to Knowledge 19 (No. 241): 1–262, pl. 1–21. 1872.

EXPLANATION OF PLATES 14–17

(All figures × 750)

PLATE 14

Fig. 1. *Aphanothece microscopica* Naegeli. Only a few cells of the colony drawn in detail.

Fig. 2. *Microcystis aeruginosa* Kuetzing. Pseudovacuoles will be observed in these cells.

Fig. 3. *Gloeocapsa conglomerata* Kuetzing.

Fig. 4. *Aphanocapsa grevillei* (Hassall) Rabenhorst. Plants in different stages of division.

Fig. 5. *Phormidium crouani* Gomont.

Fig. 6. *Phormidium retzii* (C. A. Agardh) Gomont.

Fig. 7. *Chamaesiphon incrustans* Grunow.

Fig. 8. *Oscillatoria tenuis* Agardh. Apical portions of trichomes showing variations within the species. (Figs. after M. Forbes.)

Fig. 9. *Phormidium autumnale* (Agardh) Gomont.

Fig. 10. *Gomphosphaeria aponina* Kuetzing var. *delicatula* Virieux. Portion of a mature colony.

Fig. 11. *Aphanothece pallida* (Kuetzing) Rabenhorst. Several plants in various stages of division.

Fig. 12. *Chroococcus turgidus* (Kuetzing) Naegeli.

PLATE 15

Fig. 13. *Microcoleus vaginatus* (Vaucher) Gomont.

Fig. 14. *Stigonema turfaceum* (Berkeley) Cook.

Fig. 15. *Scytonema carolinianum* sp. nov.

Fig. 16. *Scytonema carolinianum* sp. nov. A portion of a filament showing agglutination of the false branches.

Fig. 17. *Scytonema crustaceum* Agardh.

Fig. 18. *Cylindrospermum muscicola* Kuetzing.

PLATE 16

Fig. 19. *Schizothrix friesii* (Agardh) Gomont.

Fig. 20. *Cylindrospermum alatosporum* F. E. Fritsch. Two trichomes, one with a hypnogonidium in its early stage of development, the other with a nearly mature resting cell, and a series of five drawings showing successive stages in the germination of a hypnogonidium.

Fig. 21. *Nostoc ellipsosporum* (Desmaz.) Rabenhorst var. *minimum* var. nov.

Fig. 22. *Lynbya martensiana* Meneghini.

Fig. 23. *Nostoc punctiforme* (Kuetzing) Hariot. A single small colony.

Fig. 24. *Schizothrix aikenensis* (Wolle) comb. nov.

Fig. 25. *Anabaena parva* sp. nov. Several trichomes, each with developing hypnogonidia.

Fig. 26. *Cylindrospermum trichotospermum* Frémy.
Fig. 27. *Lyngbya aestuarii* (Mertens) Liebman.

PLATE 17

Fig. 28. *Tolypothrix delicatula* sp. nov. Portion of a single branching filament, and two heterocysts of different forms.
Fig. 29. *Hapalosiphon welwitschii* W. and G. S. West.
Fig. 30. *Calothrix genuflexa* sp. nov. An entire filament, drawn in sections, epiphytic on a filament of *Lyngbya* sp.
Fig. 31. *Tolypothrix rechingeri* (Wille) Geitler.
Fig. 32. *Stigonema hormoides* (Kuetzing) Bornet and Flahault.
Fig. 33. *Anabaena torulosa* (Carm.) Lagerheim.
Fig. 34. *Anabaena laxa* (Rabenhorst) A. Braun.
Fig. 35. *Calothrix braunii* Bornet and Flahault var. *maxima* var. nov.

PLATE 14

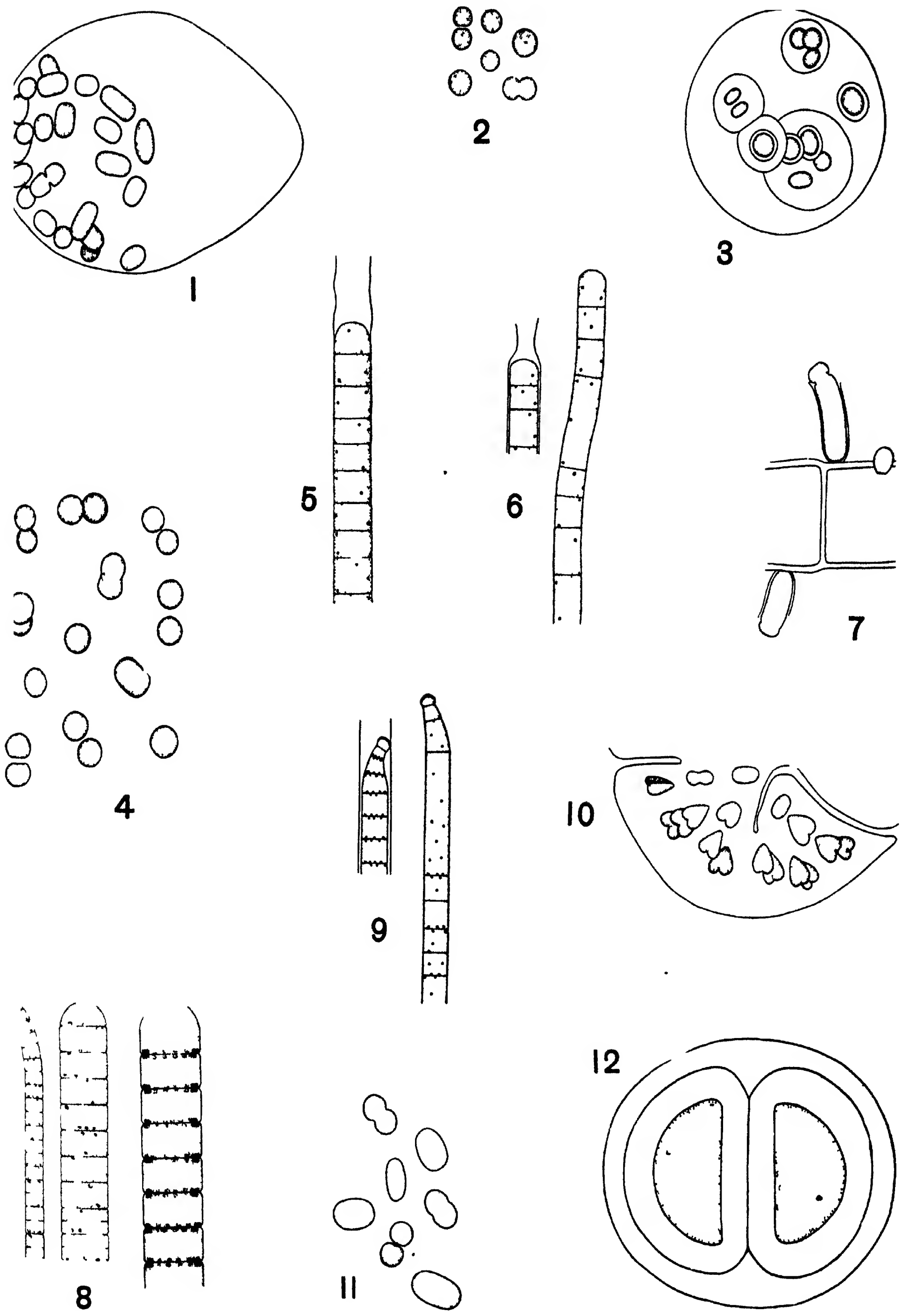


PLATE 15

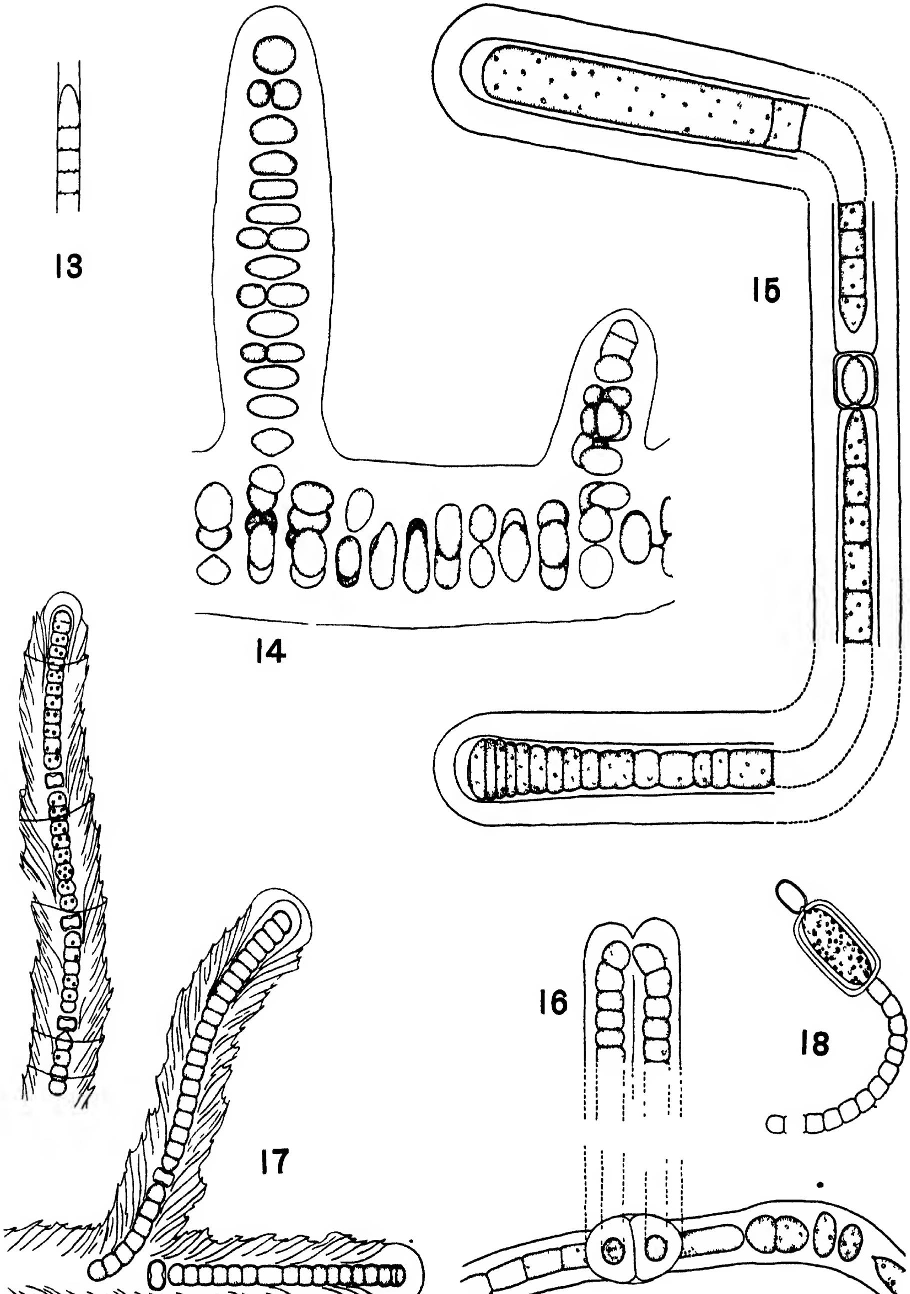


PLATE 16

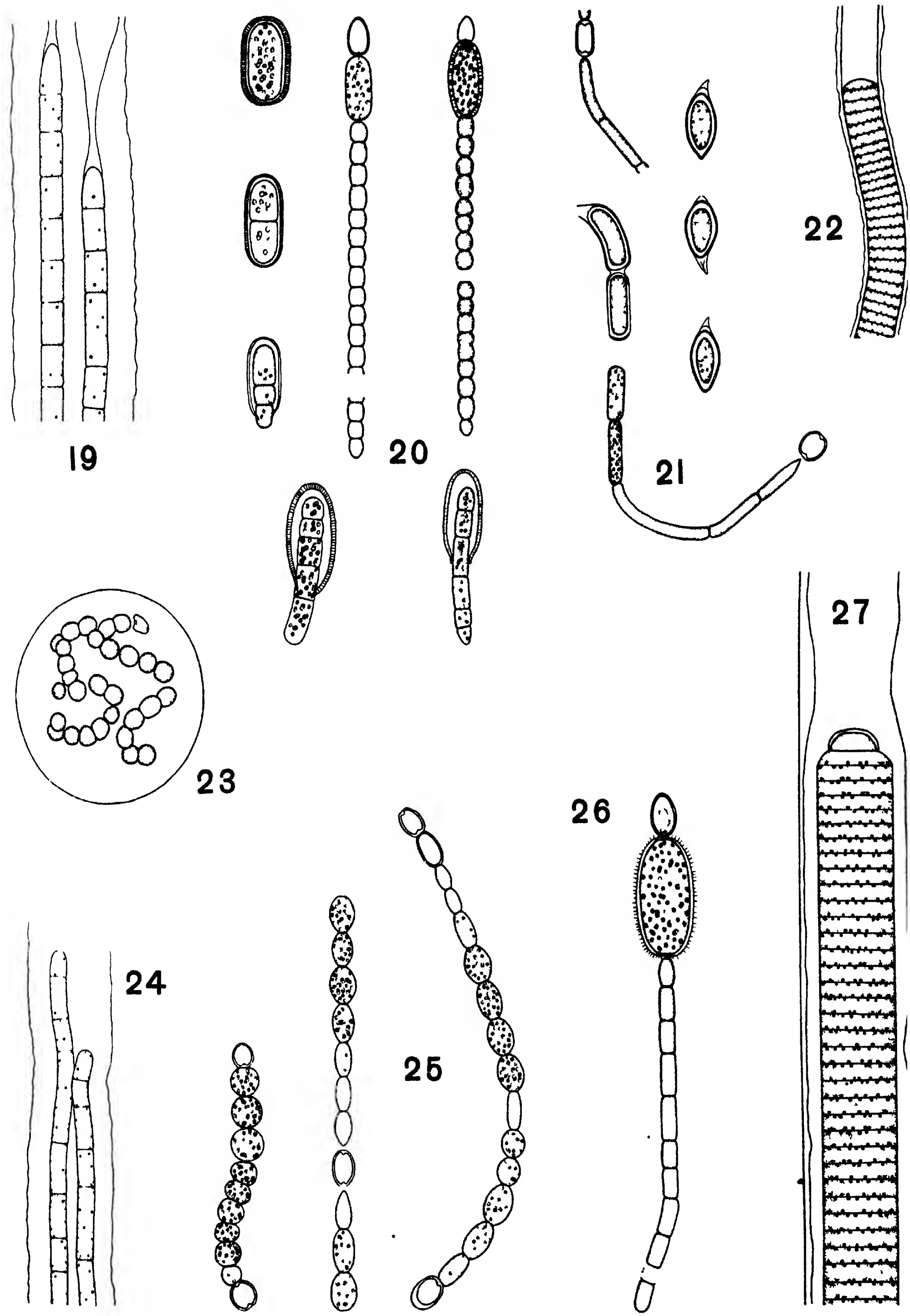
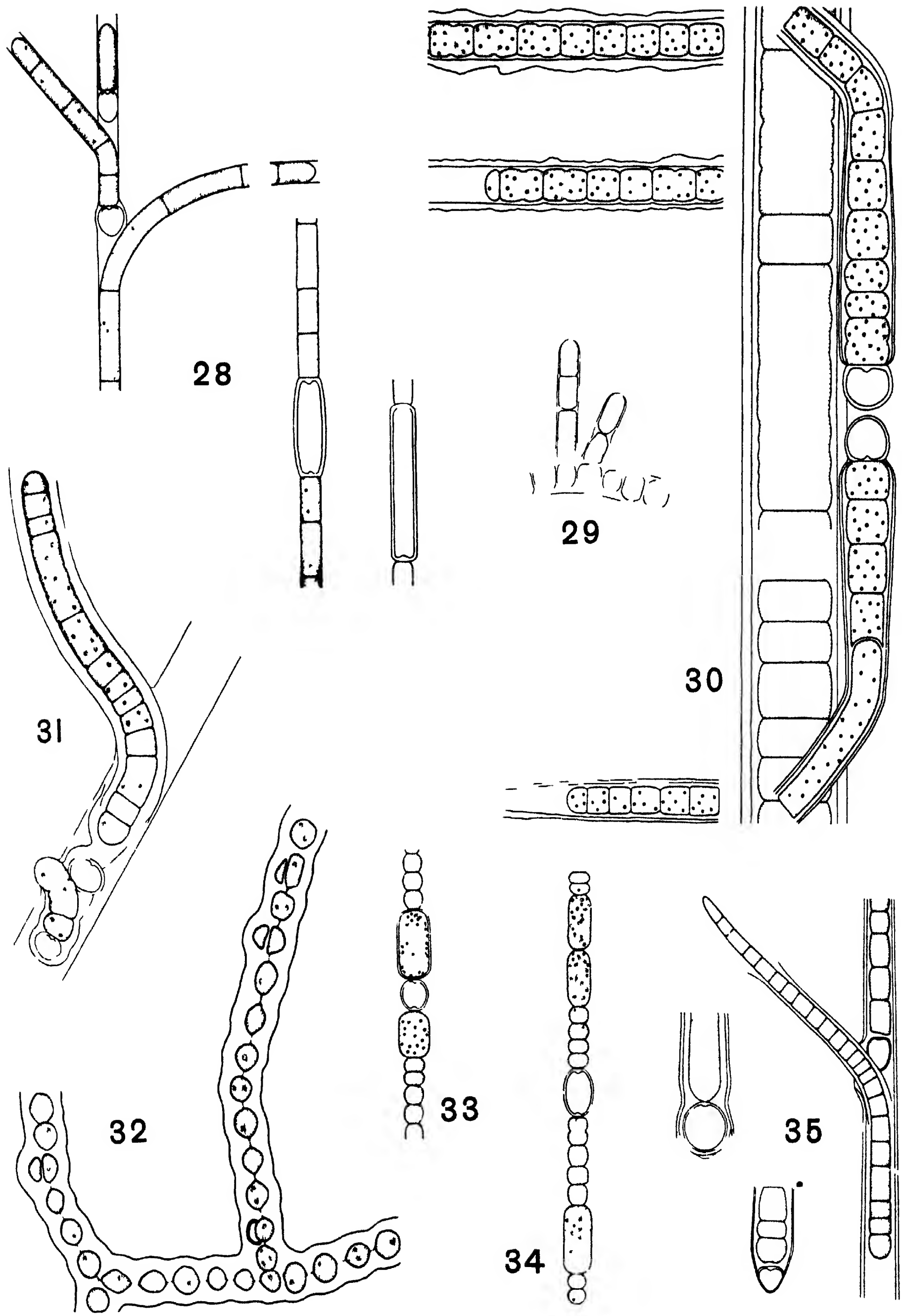


PLATE 17



CORDYCEPS FROM THE MOUNTAINS OF NORTH CAROLINA AND TENNESSEE[1]

By E. B. Mains

Plates 18–21

The mountainous area of western North Carolina and eastern Tennessee appears to be very favorable for species of Cordyceps. In this paper, 20 species are reported. In 1910 Seaver (14) treated the genus for North America, recording 18 species. Thirteen species were reported for the United States. Several of these have since been shown to be synonymous and several new species have been described, the number of species now recorded for the United States being 21. In this paper 6 additional species are reported, of which 3 have not previously been described. Thus out of a total of 27 species for the United States 20 are here recorded for the mountains of North Carolina and Tennessee. *Cordyceps gracilis* Mont. & Dur., *C. clavulata* Schw., *C. acicularis* Rav., and *Ophiocordyceps macularis* Mains are not recorded but doubtless occur. *Cordyceps palustris* Berk. & Br., *C. insignis* Cooke & Rav., and *C. armeniaca* Berk. & Curt. are based on early collections and are known only from the types or have been rarely collected.

The writer's attention was directed to this area by the collections of Dr. A. H. Smith who spent parts of the summers of 1937 and 1938[2] with Professor L. R. Hesler studying the Agaricaceae of the Great Smoky Mountains National Park. He obtained many collections in other groups, among which were 50 belonging to 13 species of Cordyceps, one proving to be new.

Through the kindness of Dr. David H. Linder it has also been possible to study the collections of Professor Roland Thaxter now in the Farlow Herbarium of Harvard University. In 1887 and 1896, Professor Thaxter collected at Cranberry, North Carolina, and Burbank, Tennessee. He obtained many interesting collections of Cordyceps but

[1] Paper from the Department of Botany and the Herbarium of the University of Michigan.

[2] Dr. Smith's studies were supported by funds from the Horace H. Rackham School of Graduate Studies of the University of Michigan.

apparently did not publish the results of his studies. Thirteen species have come to the writer's attention, three having been recently described by Petch (13) and one being described as new in this paper.

Collections have also been received from Professor W. C. Coker of the University of North Carolina and Professor L. R. Hesler of the University of Tennessee which have given a number of additional records and one new species.

KEY TO THE SPECIES

1.	Perithecia superficial, free or crowded, sometimes adhering, not embedded in a stroma	2
1.	Perithecia embedded in a stroma, sometimes only partially	10
2.	Clavae lacking	3
2.	Clavae present	4
3	Perithecia developing from a mycelial mat on mature moths	*C Sphingum* p 121
3	Perithecia developing from the apex of a buried pseudostalk	*C subsessilis* p. 122
4.	Clavae very short, 1.5–2.5 mm, on spiders	*C Thaxteri* p 120
4	Clavae longer, on insects	5
5.	Perithecia crowded, often adhering in groups	6
5	Perithecia free, scattered	7
6.	Clavae vinaceous-fawn, asci clavate, ascospores fusoid, entire, on larvae of beetles	*Ophiocordyceps macularis*[3]
6	Clavae ochraceous, asci cylindric, ascospores filiform, segmenting, on larvae of beetles	*C variabilis* p 123
7.	Clavae yellowish white, soft, variable, on mature moths	*C Sphingum* p 121
7	Clavae orange, ochraceous, or brownish black	8
8	Clavae brownish black, club-shaped, on larvae of beetles	*C Ravenelii* p 122
8	Clavae grayish brown, filiform, on larvae of beetles	*C superficialis* p 122
8	Clavae ochraceous orange, filiform	9
9.	Asci cylindric, part spores 20–50 μ	*C. michiganensis* p 123
9	Asci clavate, spores not segmenting	*C acicularis*[3]
10	Parasitic on fungi (*Elaphomyces* spp)	11
10	Parasitic on insects	13
11.	Clavae club-shaped, part spores 3–4 x 2 μ	*C ophioglossoides* p 119
11.	Clavae capitate	12
12.	Part spores 3–5 x 1 5–2 μ	*C. intermedia* p 120
12.	Part spores 25–50 x 4 μ	*C. capitata* p 119
13.	Stromata developing as cushions or irregular patches on clavae	14
13	Stromata regularly distributed on clavae	16
14.	Stromata forming irregular patches on large clavae, 40–50 x 10–15 mm., on larvae of beetles	*C. Melolanthae* p 124

[3] This species has apparently not been collected in the area under consideration, but doubtless occurs.

14. Stromata forming cushions laterally placed on slender clavae............ 15
15. Fertile stromata black, on ants.........................*C. unilateralis* p. 124
15. Fertile stromata orange, on larvae of beetles..............*C. viperina* p. 124
16. Clavae capitate.. 17
16. Clavae club-shaped, cylindric or filiform................................ 19
17. Heads hemispherical, ochraceous orange, on flies.......*C. dipterigena* p. 127
17. Heads hemispherical, black, on ants..................*C. unilateralis* p. 124
17. Heads spherical or ovoid... 18
18. Clavae yellow, asci cylindric, ascospores filiform, segmenting, on wasps *C. sphecocephala* p. 127
18. Clavae with yellow stalk, mahogany red head, ascospores filiform, segmenting, on lepidopterous larvae..................................*C. gracilis*[3]
18. Clavae reddish brown, asci clavate, ascospores fusoid, entire, on wasps *C. Smithii* p. 127
19. Apices of clavae sterile, fertile portion cylindric........................ 20
19. Apices of clavae covered with perithecia, fertile portion club-shaped...... 21
20. Perithecia projecting one-third from a yellowish stroma, on lepidopterous larvae..*C. elongata* p. 126
20. Perithecia completely embedded in brownish stromata, on larvae of beetles *C. stylophora* p. 126
21. Clavae 3–4 mm. long, on scale insects........................ *C. clavulata*[3]
21. Clavae much larger, on other insects.................................. 22
22. Fertile portion black, on Cicada.......................*C. Hesleri* p. 125
22. Fertile portion orange, on lepidopterous pupae.........*C. militaris* p. 125

CORDYCEPS OPHIOGLOSSOIDES (Ehrh.) Link.

On *Elaphomyces* spp. Great Smoky Mountains National Park, Aug. and Sept. 1937 and 1938, A. H. Smith, common; Highlands, N. C., Sept. 1937, Aug. 1938, A. H. Smith (7503, 7521, 10390).

This is the most abundant and widespread species parasitizing *Elaphomyces*. The clavae are greenish brown to greenish black, club-shaped, the fertile portion cylindric or narrowly ellipsoid. The clavae are usually attached to the tubers by long yellow rhizomorphs. The perithecia are entirely embedded and are narrowly ovoid. The asci are cylindric, slightly narrowing at the apex and toward the base, 270–360 x 7–8 μ. The ascospores are filiform, nearly as long as the asci, multiseptate, early separating into the component cells which are short cylindric, 3–4 x 2 μ. There are specimens in the Farlow Herbarium collected by R. Thaxter at Burbank, Tennessee.

CORDYCEPS CAPITATA (Holm.) Link.

On *Elaphomyces* sp. Great Smoky Mountains National Park, Keener House, Aug. 3, 1938; Le Conte, Aug. 23, 1938, A. H. Smith (9666, 10475).

The clavae are olivaceous to greenish black, capitate, and are seated directly on the host. The heads are spherical and the perithecia are embedded. The asci are usually described as cylindric but in these specimens are narrowly fusoid, 320–390 μ long and 14–16 μ wide, narrowing at each end to 6–8 μ. The ascospores are multiseptate, filiform, nearly as long as the asci, 4 μ wide, tapering at each end to 2 μ. The ascospores early break into the component cells which are slightly fusoid, (16) 26–50 μ long, and 4 μ wide, narrowing to 2–2.5 μ at the ends. The end walls are somewhat thickened. This species varies somewhat in the size of the asci and specially of the part spores. In some collections the latter are much smaller, 14–30 x 2–3 μ, at one time being recognized as a separate species, *Cordyceps canadensis* Ellis and Everh.

CORDYCEPS INTERMEDIA Imai.

On *Elaphomyces* sp. Cades Cove, Great Smoky Mountains National Park, Aug. 18, 1938, A. H. Smith (10336).

This collection is very interesting. In its gross morphology and habit, it resembles *Cordyceps capitata*. The clavae are capitate and arise directly from the tubers. In the character of asci and ascospores it approaches *C. ophioglossoides*. The asci are nearly cylindric, 420–480 x 6 μ, narrowing only slightly toward the apex. The spores are filiform, nearly as long as the asci, multiseptate, the part spores short-cylindric, 3–5 x 1.5–2 μ. This species was described by Imai (3) from Japan and it is interesting that it should be found in the eastern United States. The clavae are smaller than those described from Japan but *C. capitata* shows a similar variation. It is possible that this species may be a hybrid between *C. capitata* and *C. ophioglossoides*.

CORDYCEPS THAXTERI sp. nov. Plate 18, figs. A–D

Clavis 2–10, pallide brunneis, 1.5–2.5 mm. altis, 150–180 μ crassis, sursum incrassatis 180–300 μ; peritheciis paucis, superficialibus, ovoideis, 960–1200 x 300–360 μ; ascis cylindraceis, 600–780 x 6–7 μ; ascosporis filiformibus, 1–1.5 μ crassis, multiseptatis, septis 6–10 μ distantibus; conidiosporis catenulatis, hyalinis, obovoideis, 4–11 x 2 μ.

In araneis, Cranberry, N. C., July–Aug. 1887, R. Thaxter (Farlow Herb. 4123, specimen typicum); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 4122A).

This species is unusual in a number of respects. Collection 4123 contains approximately 70 small spiders covered with cottony masses of mycelium which attach the hosts to leaves. Each specimen bears

several pale buff clavae which are 1.5–2.5 mm. long, 150–180 μ thick below, usually enlarging above to 180–300 μ. The majority of the clavae bear only conidia which are produced in such quantity that they are often covered with large floccose masses. The conidia are catenulate and are developed on brown, slightly roughened, clavate cells which cover the clavae. The conidia are narrowly obovoid, 4–11 x 2 μ. It apparently is *Isaria arachnophila* Ditmar. Petch (8) has shown that the fungus most commonly reported as *Isaria arachnophila* is *Gibbelula arenarum* and that (9) the latter is the conidial stage of *Torrubiella Gibbelulae* Petch. Petch (8) has transfered *Isaria arachnophila* to *Hymenostilbe.*

Only a few, 1–4, perithecia develop on a clava. They are orange, conic-ovoid, and very large in proportion to the clavae, 960–1200 x 300–360 μ. The asci also are unusual for their size, 600–780 x 6–7 μ. The ascospores are nearly as long as the asci, 1–1.5 μ in width, multiseptate with the cells 6–10 μ long, tardily breaking into part spores.

The type is in the Farlow Herbarium of Harvard University, and in the Herbarium of the University of Michigan.

CORDYCEPS SPHINGUM (Tul.) Berk. & Curt.

On mature moths, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4054); Cranberry, N. C., Aug. 1896, R. Thaxter (Farlow Herb. 4053); Burbank, Tenn., R. Thaxter (Farlow Herb. 6192); Pretty Hollow, Haywood Co., N. C., Aug. 11, 1936, Lane Barksdale.

This is a very variable species which has been described under a number of names (10). In the above collections, the mature moths are covered with a matted mass of mycelium which also fastens them to the substratum. Some specimens are sterile or bear only conidia. Others lack clavae, and perithecia are produced on the matted mycelium covering the moth. When clavae are produced they vary considerably in number, form and size. They are soft, whitish, and often reach 10 mm. in length. The perithecia are scattered or crowded in groups. They are entirely superficial or immersed up to one-third of their height. They are brown, ovoid, 300–540 x 180–300 μ and contain cylindric asci 270–300 x 4 μ with filiform, multiseptate ascospores nearly as long as the asci, the part spores 4–6 x 1 μ.

There is some question concerning the first name which was applied to the perithecial stage. The Tulasnes (15) under the name *Torrubia sphingum* described perithecia from North American specimens. They cited *Isaria sphingum* Schw. for the conidial stage and *Akrophyton*

tuberculatum Lebert for the perithecial stage. Petch (10) concluded that Lebert's name should be used. Later he (10) has expressed doubt whether Lebert's name should apply to the North American fungus. It seems best for the present to employ the name used by both Schweinitz and the Tulasnes based on North American collections.

CORDYCEPS SUBSESSILIS Petch.

On larvae of beetles in rotten wood, Cranberry, North Carolina, 1887, R. Thaxter (Farlow Herb. 6155); Burbank, Tennessee, Aug. 1896, R. Thaxter (Farlow Herb. 6145).

Petch (13) described this species from the specimens cited above. The material is scanty and gives one the impression of poorly developed individuals. Petch states that it is doubtful whether the species should be described as a Cordyceps or a Torrubiella. He states that the species have a pseudostalk which may represent strands of mycelium bringing the fungus to the surface of the wood where perithecia are produced. They are described as free, crowded, narrowly flask-shaped, amber, 1–1.5 x 0.33–0.44 mm., containing cylindric asci 240 x 5 μ and part spores 3–7 x 1 μ.

CORDYCEPS RAVENELII Berk. & Curt.

On larvae of a beetle, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4050); Great Smoky Mountains National Park, Cades Cove, Aug. 18, 1938, A. H. Smith (10327).

These specimens have brownish-black, club-shaped clavae, 5.5 and 6.5 cm. long, 1.5 mm. thick, the fertile portion 2–2.5 mm. thick, the apices sterile, acute. The perithecia are superficial, free, closely grouped on the upper third, broadly ovoid, 400–480 x 300 μ. The asci are cylindric-clavate, 180–240 x 6–8 μ. The ascospores are cylindric, 160–190 x 2 μ, overlapping somewhat in the asci, multiseptate, the cells 22–30 μ long, tardily breaking into one-celled part spores.

This resembles *C. superficialis* in many characters. It differs in the more robust development, the darker color, and smaller more crowded perithecia. Petch (11) considers *C. Ravenelii* a synonym of *C. acicularis*. However, the latter has slender, ochraceous clavae which serve to distinguish it.

CORDYCEPS SUPERFICIALIS Peck.

On larvae of a beetle, Great Smoky Mountains National Park, Fighting Creek Gap, Aug. 22, 1938, A. H. Smith (10401); Highlands, N. C., Aug. 21, 1938, A. H. Smith (10395).

The clavae of these collections are grayish brown, up to 4.5 cm. long, 0.5–1.0 mm. thick. The perithecia are superficial, free, scattered and the apices of the clavae are sterile and acuminate. The perithecia are broadly ovoid to conic-ovoid, 480–600 x 420–480 μ, dark brown with grayish brown scales. The asci are cylindric, 200–270 x 6–8 μ. The ascospores are nearly as long as the asci, multiseptate, breaking into one-celled, cylindric segments, 16–32 x 1.5–2 μ.

This species was described by Peck (7) from New York. Seaver (14) placed it in synonymy with *Cordyceps acicularis*. The latter, however, has ochraceous clavae, clavate asci, and fusoid ascospores (5). It has been reported (5, 6) from New York, Michigan, Massachusetts, Connecticut, and Ontario.

CORDYCEPS MICHIGANENSIS Mains.

On larvae of beetles, Cullowhee, N. C., 1887, R. Thaxter (Farlow Herb. 6140); Great Smoky Mountains National Park, Keener House, Aug. 3 and 22, 1938, A. H. Smith (9667, 10397).

These specimens have several short slender clavae arising from the larvae. The clavae are ochraceous-orange, up to 3.5 cm. long, 1 mm. thick, the apices sterile and acuminate. The perithecia are superficial, free, scattered irregularly over the middle portion of the clavae. The asci are cylindric, up to 240 μ long and 6 μ wide. The ascospores are filiform, nearly as long as the asci, multiseptate, the cells 20–50 x 1.5 μ, somewhat tardily fragmenting. This species resembles *Cordyceps acicularis* in some respects, the latter differing in fewer, longer clavae, clavate asci, and fusoid ascospores. *Cordyceps michiganensis* has previously been reported (5, 6) from Michigan and Ontario.

CORDYCEPS VARIABILIS Petch. Plate 20, fig. D.

On larvae of beetles in rotten logs, Burbank, Tenn., Aug. 7, 1886, R. Thaxter (Farlow Herb. 6144); Great Smoky Mountains National Park, A. H. Smith; Indian Creek, Sept. 5, 1937 (7393), Aug. 14, 1938 10169), Sept. 2, 1938 (10834); Coon's Cove, Aug. 8, 1938 (9861); Alum Cove Trail, Sept. 1, 1938 (10772).

This species was recently described by Petch (13) from specimens from New York, Tennessee, Wisconsin, and Maine. As the name implies it is very variable. The specimens listed above have ochraceous clavae, 3–8 mm. long, 0.5 mm. thick. The perithecia are in small groups on one side of the clavae at various distances below the apices. They are superficial, adhering at their bases and sometimes for about

half their height. The asci are cylindric-fusoid, 200–330 x 6–8 μ, and the ascospores filiform, breaking into cylindric segments, 6–8 x 1.5–2 μ.

This species shows some resemblance to *Cordyceps viperina*. The latter has its perithecia embedded in a stroma.

CORDYCEPS VIPERINA Mains

On the larvae of beetles in rotten logs, Great Smoky Mountains National Park, Alum Cove Trail, Aug. 25, 1938, A. H. Smith (10543).

This species was recently described (6) from specimens collected in Nova Scotia, New York, Ontario, and British Honduras. This collection has ochraceous-orange clavae, 5–12 mm. long, 0.5 mm. thick. The perithecia are embedded in a pulvinate stroma which develops on one side of the clava some distance below the apex. The asci are cylindric-clavate, 200–250 x 8–10 μ, narrowing to 4–6 μ at the apex. The ascospores are filiform, multiseptate, early breaking into one-celled cylindric part spores, 6–8 x 2 μ.

CORDYCEPS UNILATERALIS (Tul.) Sacc. Plate 20, figs. A–C.

On ants, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4057); Cullowhee, N. C., June 15, 1896, R. Thaxter (Farlow Herb. 6161), June 1887 (Farlow Herb. 6190).

This interesting species produces clavae varying considerably in length, from a few mm. up to 20 mm. or more. They are very slender, black or brownish black, usually arising between the head and thorax. The perithecia are produced in pulvinate black stromata which may develop at various places along the clavae or occasionally may be terminal. Usually only a single stroma is produced although sometimes two or more may develop. The perithecia are fusoid-ellipsoid, 275–360 x 120–156 μ, entirely embedded in the stroma. The stroma between the upper portions of the perithecia is shrunken, giving the surface a verrucose appearance. The asci are clavate, 150–200 x 8–10 μ, and the ascospores, cylindric-fusoid, 132–162 x 2.5–3 μ, the ends acuminate. They are multiseptate, the septa 12–14 μ apart, not fragmenting.

CORDYCEPS MELOLANTHAE (Tul.) Sacc.

On large white grubs of beetles, Great Smoky Mountains National Park, A. H. Smith: Laurel Falls Trails, Aug. 8, 1938 (9893); Cades Cove, Aug. 16, 1938 (10245).

Each specimen has a single, pale ochraceous clava. They are irregularly clavate, somewhat furcate, 4.5–5 cm. long, 6 mm. wide below,

enlarging above to 12–15 mm. The perithecia are embedded in scattered irregular patches of stroma on the upper portion of the clavae. The asci are fusoid-cylindric, 180–240 x 6–7 μ, the ascospores filiform, nearly as long as the asci, multiseptate, soon breaking into cylindric segments, 4–6 x 1.5 μ.

This species has usually been reported as *Cordyceps herculea* (Schw.) Sacc. This name was based on *Sphaeria herculea* Schw. which Lloyd (4) has shown is a Gasteromycete *Cauloglossum transversarium* (see also 5, 12).

CORDYCEPS HESLERI sp. nov. Plate 19, figs. A & B.

Clava atra, 3 cm. longa, 5–7 mm. crassa; stipite griseo, 2 cm. longo; peritheciis fusoideo-ellipsoideis, 780–960 x 360–420 μ, immersis; ascis cylindriceis, 300–360 x 6–7 μ; ascosporis filiformibus; articulis ascosporarum, 3–4 x 1.5–2 μ.

In Cicada, Great Smoky Mountains National Park, Cades Cove, Blount Co., Tenn., Oct. 17, 1937, L. R. Hesler (Herb. Univ. Tenn, 10939).

The clava of this specimen is club-shaped, 3 cm. long (4.5 cm. when fresh). The stalk is 20 mm. long, 5 mm. thick, gray tinged with olive and yellowish at the base. The fertile portion is black and slightly thicker, 7 mm. The perithecia are entirely embedded, fusoid-ellipsoid, 780–960 x 360–420 μ. The upper layer of the stroma is made up of dark compact hyphae, the lower layer of hyaline, loosely woven hyphae. The upper layer in the dried specimen has shrunken and conforms to the outlines of the upper portions of the perithecia, giving the stroma a very rough appearance. The asci are cylindric, 300–360 x 6–7 μ, the ascospores filiform, nearly as long as the asci. The spores break into partspores 3–4 x 1.5–2 μ. The type is deposited in the Herbarium of the University of Michigan.

According to Petch (12) the various species described on Cicada are *Cordyceps sobolifera*. This species differs in a number of respects from *C. Hesleri*.

CORDYCEPS MILITARIS (L.) Link.

On pupae of lepidopterous insects, Great Smoky Mountains National Park, Aug. and Sept. 1937 and 1938, A. H. Smith, common; Highlands, N. C., Sept. 9, 1937, A. H. Smith (7506).

This is the most common species of Cordyceps. The orange, club-shaped clavae vary considerably in size, apparently correlated with the

size of the pupae from which they develop. The perithecia at maturity are only partially embedded in a soft stroma. The asci are cylindric, 400 μ or more long, 4 μ wide, the ascospores filiform, nearly as long as the asci, multiseptate, the part spores cylindric, 3–6 x 1 μ.

CORDYCEPS ELONGATA Petch.

On pupae and larvae of a moth *Apatela americana*, Cullowhee, N. C., R. Thaxter (Farlow Herb. 6277); Burbank, Tenn., Sept. 1887, R. Thaxter (Farlow Herb. 6134); Cranberry, N. C., 1887, R. Thaxter (Farlow Herb. 6135); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 3906).

The clavae are 5–11 cm. long, slender, the stalks 0.5–1.5 mm. thick, light brown, the fertile portions cylindric, terete, 1.5–3.5 cm. long, slightly wider than the stalks, 1.5–2.5 mm. The stroma between the reddish brown perithecia is pale yellow. The clavae often terminate in a short, acuminate, sterile apex. The perithecia are embedded except for the upper third. They are ovoid, 420–480 x 240–276 μ. The asci are fusoid-cylindric, 240–300 x 7–8 μ, narrowing slightly toward the ends. The ascospores are filiform, nearly as long as the asci, 2–2.5 μ wide, slightly overlapping in the asci, multiseptate, not breaking into part spores. Three of the above collections were cited by Petch (13) in his description of the species. He also records a collection from Maine. Petch suggests that *Hirsutella gigantea* Petch is the conidial stage.

CORDYCEPS STYLOPHORA Berk. & Br.

On larvae of beetles in rotten logs, Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 4055); Great Smoky Mountains National Park, Alum Cave Trail, Aug. 22 and 26, 1938, A. H. Smith (10544, 11120).

These collections are immature. The clavae are grayish-brown, filiform to narrowly cylindric, up to 2.5 cm. long, the stipe 0.8 mm. thick, the cylindric stromata 1 mm. thick, the apices sterile, up to 5 mm. long, acuminate. This species is often collected in various degrees of maturity. Mature specimens have perithecia entirely embedded, the asci cylindric, 180–300 x 7–8 μ, the ascospores nearly as long as the asci, filiform, breaking into segments 6–10 x 2 μ. This species is fairly rare. It was first collected by Ravenal in South Carolina about 1857 (1). The next reported collection was by G. H. Hicks from Michigan in 1892 (2). Since 1934 it has been reported from Michigan and New York (5, 6).

CORDYCEPS DIPTERIGENA Berk. & Br. Plate 21, figs. A & B.

On flies, Cranberry, N. C., Aug. 1887, R. Thaxter (Farlow Herb. 4052, 5086, 6233c, 5077, Rel. Farl. 612); Burbank, Tenn., Aug. 1896, R. Thaxter (Farlow Herb. 6192).

The flies are covered with a yellowish mycelium by which they are attached to the substratum. One, two, and sometimes four clavae develop. These are 4–6 mm. long, capitate, the head hemispherical or turbinate, ochraceous-orange, punctate with brown ostioles, 1.5–2 mm. across. The stalks are slender, light buff. The perithecia are entirely embedded, directed upward with ostioles on the upper surface of the head, narrowly ovoid or fusoid-ovoid, 720–1000 x 240–540 μ. The asci are narrowly cylindric, 300–450 x 4–6 μ, the ascospores nearly as long as the asci, filiform, multiseptate, soon breaking into fusoid segments 8–12 x 1.5 μ.

This species has been rarely collected in the United States. There is a sterile specimen in the Mycological Collections of the United States Bureau of Plant Industry from Mississippi. Collections from Puerto Rico, Costa Rica, and Trinidad have been seen.

CORDYCEPS SPHECOCEPHALA (Klatzsch) Massee. Plate 21, figs. C & D.

On mature wasps, Great Smoky Mountains National Park, A. H. Smith: Elkmont, Sept. 7, 1937 (7478); Keener House, Aug. 3 and 8, 1938 (9664, 9938); LeConte, Aug. 17, 1938 (10305); Indian Creek, Sept. 5, 1937, and Aug. 14, 1938 (7399, 10176).

The clavae are capitate, 2–10 cm. long, the stalks light brown, slender, 0.5 mm. or less thick, the heads yellow, ovoid or oblong, 2–8 x 1.5–3 mm. The dried heads are irregularly longitudinally wrinkled. The perithecia are embedded except for the slightly projecting ostioles. The asci are cylindric, 240–300 x 5–6 μ, the ascospores filiform, nearly as long as the asci, multiseptate, breaking into part spores 8–12 x 1.5–2 μ.

This species has previously been reported from North America only from the West Indies. There is also a specimen in the Mycological Collection of the United States Bureau of Plant Industry from Florida.

CORDYCEPS SMITHII sp. nov. Plate 19, figs. C & D.

Clavis rubro-brunneis, 4–5 cm. longis, stipitibus 0.5 mm. crassis, capitibus conoideis vel ovoideis 4–5 x 1.5–2 mm.; peritheciis immersis, ovoideis, 240–260 x 150–160 μ; ascis clavatis, 90–120 x 8–10 μ; ascosporis fusoideis, 60–100 x 2.5–3 μ, multiseptatis.

In vespis, Great Smoky Mountains National Park, Keener House, Aug. 22, 1938, A. H. Smith (10396).

This specimen has two clavae arising from a wasp. They are reddish-brown, capitate, 4–5 cm. long, the stalks 0.5 mm. thick and the conic-ovoid heads 4–5 x 1.5–2 mm. The perithecia are embedded in the head and are ovoid, 240–260 x 150–160 μ. The asci are clavate, 90–120 x 8–10 μ, the ascospores fusoid, 60–100 x 2.5–3 μ, acuminate at each end and overlapping in the ascus. The spores are obscurely multiseptate and are not broken into part spores. The type specimen is deposited in the Herbarium of the University of Michigan.

Petch (12) in his discussion of the species of Cordyceps infecting bees and wasps described only one species with clavate asci and fusoid ascospores, *C. Humberti*. The latter, however, differs considerably in other respects from the above.

HOST INDEX

LITERATURE CITED

(1) BERKELEY, M. J. On some entomogenous Sphaeriae. Journ. Proc. Linn. Soc. 1: 157–159. 1857.

(2) ELLIS, J. B., AND EVERHART, B. M. The North American Pyrenomycetes. 793 p. Newfield, N. J. 1892.

(3) IMAI, SANSHI. On a new species of Cordyceps parasitic on Elaphomyces in Japan. Proc. Imp. Acad. Japan 10: 677–679. 1934.

(4) LLOYD, C. G. Letter no. 47, note 98. 1913.

(5) MAINS, E. B. The genera Cordyceps and Ophiocordyceps in Michigan. Proc. Amer. Phil. Soc. 74: 263–271. 1934.

(6) MAINS, E. B. A new species of Cordyceps with notes concerning other species. Mycologia 29: 673–677. 1937.

(7) PECK, C. H. Report of the Botanist. Ann. Report N. Y. St. Mus. 28: 31–88. 1875.
(8) PETCH, T. Isaria arachnophila Ditmar. The Naturalist, 1931: 247–250.
(9) PETCH, T. Gibellula. Trans. Brit. Mycol. Soc. 9: 108–128. 1924.
(10) PETCH, T. Notes on entomogenous fungi. Trans. Brit. Mycol. Soc. 16: 209–245. 1932.
(11) PETCH, T. Notes on entomogenous fungi. Trans. Brit. Mycol. Soc. 18: 48–75. 1933.
(12) PETCH, T. Notes on entomogenous fungi. Trans. Brit. Mycol. Soc. 19: 161–194. 1934.
(13) PETCH, T. Notes on entomogenous fungi. Trans. Brit. Mycol. Soc. 21: 34–67. 1937.
(14) SEAVER, F. J. Cordyceps. N. Am. Flora 3: 48–54. 1910.
(15) TULASNE, L. R., AND C. Selecta Fungorum Carpologia 3: 1–221. 1865.

EXPLANATION OF PLATES

PLATE 18

Fig. A. *Cordyceps Thaxteri.* Spider showing cottony mass of mycelium and clavae with perithecia. × 15.

Figs. B. & C. *Cordyceps Thaxteri.* Clavae bearing conidia. × 15.

Fig. C. *Cordyceps Thaxteri.* Perithecia. × 60.

PLATE 19

Fig. A. *Cordyceps Hesleri.* Cicada with clava. × 1.8.

Fig. B. *Cordyceps Hesleri.* Perithecia in stroma. × 60.

Fig. C. *Cordyceps Smithii.* Wasp with two clavae. × 1.5 (Photograph by A. H. Smith).

Fig. D. *Cordyceps Smithii.* Head with perithecia slightly projecting from the stroma. × 20.

PLATE 20

Fig. A. *Cordyceps unilateralis.* Ant with clavae, showing lateral stroma. × 4.

Fig. B. *Cordyceps unilateralis.* Head of ant with clavae showing terminal stroma. × 10.

Fig. C. *Cordyceps unilateralis.* Portion of head showing embedded perithecia. × 60.

Fig. D. *Cordyceps variabilis.* Two clavae with laterally grouped perithecia. × 10.

PLATE 21

Fig. A. *Cordyceps dipterigina.* Fly with four capitate clavae. × 5.

Fig. B. *Cordyceps dipterigina.* Portion of head of clava, showing perithecia. × 60.

Fig. C. *Cordyceps sphecocephala.* Wasp with capitate clava. × 2.

Fig. D. *Cordyceps sphecocephala.* Head showing ostioles of embedded perithecia. × 10.

PLATE 18

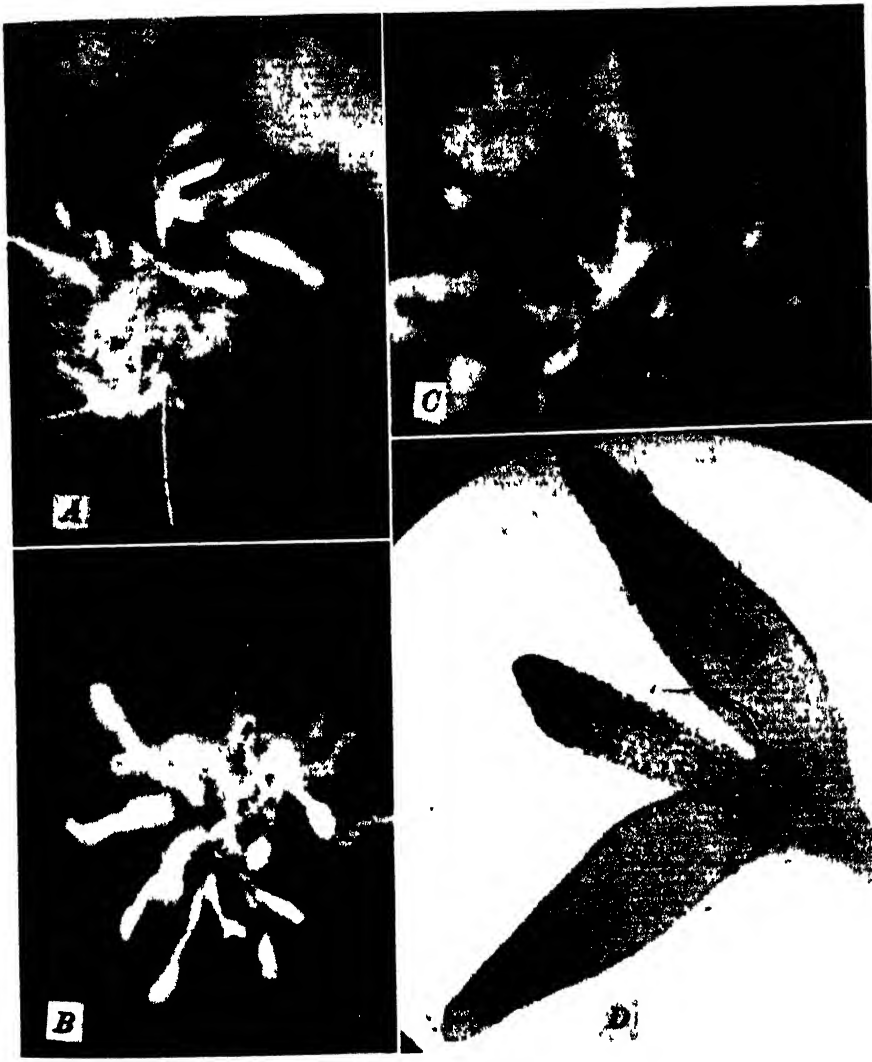
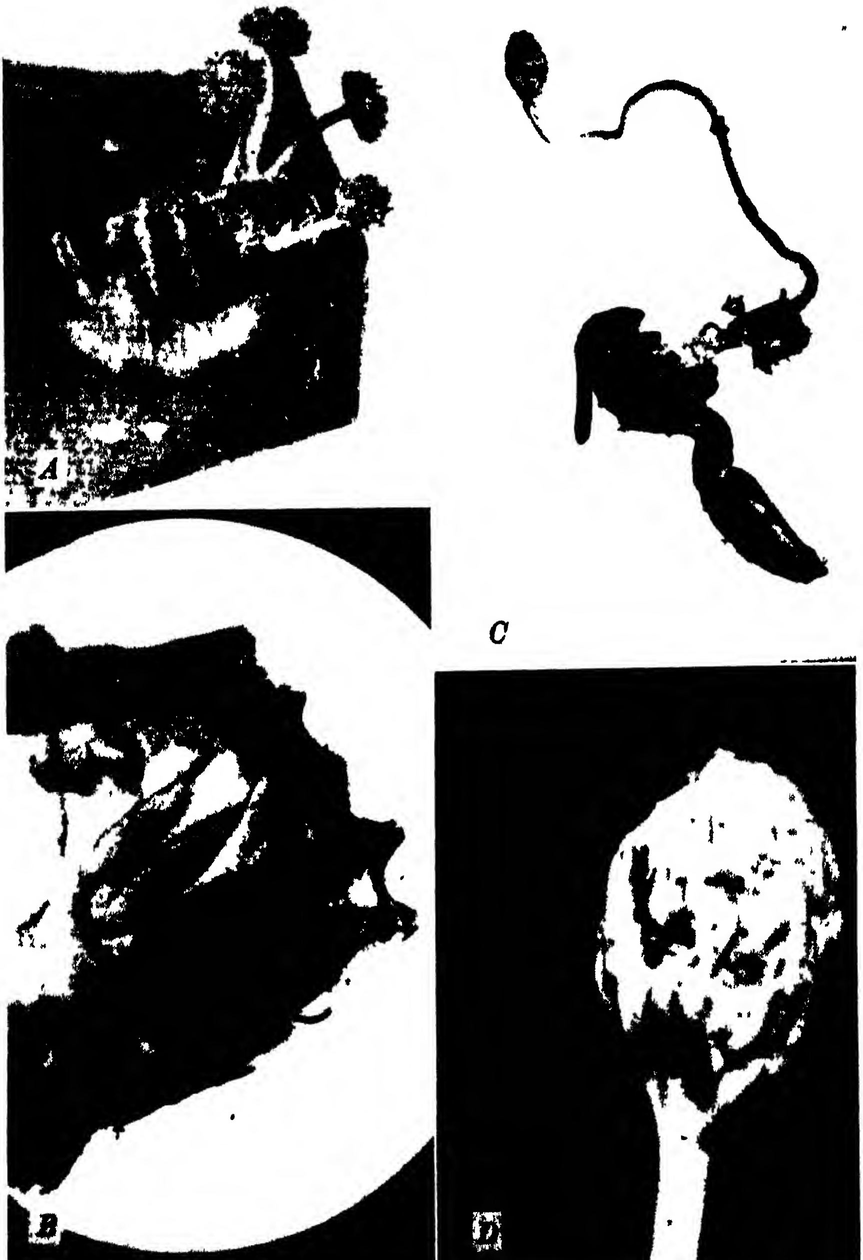


PLATE 19

PLATE 20

PLATE 21



A NEW SPECIES OF ELAPHOMYCES FROM THE GREAT SMOKY MOUNTAIN NATIONAL PARK[1]

By David H. Linder

One Text Figure

Through the kindness of Dr. E. B. Mains of the University of Michigan, the writer was enabled to study a species of *Elaphomyces* which was collected in the Great Smoky Mountain National Park by Dr. A. H. Smith. This species, represented by two fructifications, was parasitized by *Cordyceps intermedia* Imai.

The specimens of *Elaphomyces* are immediately characterized by their reddish-purple color, or "Prussian Red" of Ridgway,[2] and by their small dimensions. The specimens are further characterized by the gleba or "capillitium" which although white in a very small area at the very center, have outside of the central area a distinctly greenish-blue tinge which is made more pronounced when the specimen is wetted either with alcohol or water. Under the microscope, the fungus displays characteristics which place it in the subgenus *Malacoderma* Vitt. as recognized by Dodge[3] in his monographic study of the higher *Plectascales*. Within this subgenus the present specimens, according to descriptions, approach most closely to *Elaphomyces atropurpureus* Vitt. but a comparison with an authentic specimen of *E. atropurpureus* from Vittadini in the Patouillard Herbarium shows that there are striking differences in the color and important differences in the structure of the peridium, the peridium of the Vittadini specimen being somewhat sclerotic and about three times as thick as that of the Smoky Mountain material. Furthermore, the differences in the peridium become more marked when examined more closely under the microscope since the outer layer of the peridium of the latter specimens arises from the central layer, is composed of loosely interwoven hyphae (Fig. 1, a.) and only becomes densely organized in

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, No. 172.

[2] Ridgway, R. Color Standards and Color Nomenclature, Washington, D. C., 1912 was used wherever colors are cited within quotation marks.

[3] Dodge, C. W., Ann. Myc. 27 (3/4): 145–184, pl. 1-2, 1929.

the immediate vicinity of rootlets that are often included in the outer layer of the peridium. Unlike *E. atropurpureus*, the central layer is relatively narrow (Fig. 1, b.) and is only slightly sclerotized, while inside this median layer, and making up the bulk of the peridium is the inner peridium which is composed of large, loosely aggregated, rounded or somewhat angular cells (Fig. 1, c.) that are thin-walled and in mass "Light Pinkish Cinnamon." In contrast, then, to the thick hard layer to be found in *E. atropurpureus*, this species possesses a relatively slender, three-layered peridium of which the outer is of loosely interwoven purplish hyphae, a thin median layer of almost parallel hyphae in strands that are interwoven and are purplish, and finally an inner

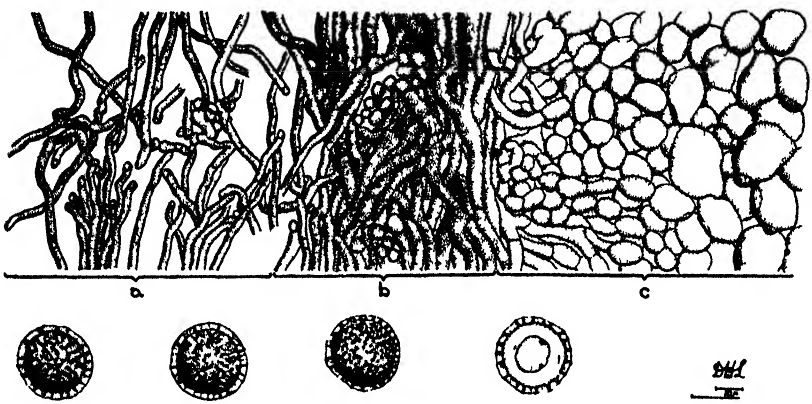


FIGURE 1

peridium of large thin-walled slightly brownish colored cells which give this layer, up to 500 μ thick, a punky texture.

Another species which approaches the present one is *E. nopporensis* Imai which was described from Japan and is the host upon which *Cordyceps intermedia* Imai was originally described. Although both the Smoky Mountain and the Japanese species of *Elaphomyces* are parasitized by the same species of *Cordyceps* and there is a resemblance between the two, they nevertheless are quite distinct. Grossly they differ in size, the American material being much smaller than the majority of the fructifications of the Japanese material, and the color more brilliant. Microscopically the two are quite distinct since the peridium of *E. nopporensis*, although relatively thin, is thicker than that of our material and, furthermore, the spores are larger, measuring (13.5)–15–16.5 μ in diameter, are considerably darker in color so that under the microscope

they appear a very deep chestnut color and the surface is densely and relatively even ornamented with close reticulations which frequently are broken into irregular, somewhat flattened, short, tooth-like echinulations. The spores of the Smoky Mountain material contrast with the others by their smaller dimensions, (6.6)–7.5–9 μ, their lighter color which is seldom darker than yellowish brown under the microscope, and, in spite of the fact that the spores are smaller than are those of *E. nopporensis*, the reticulations are approximately three or four times as coarse and the ridges or teeth, as the case may be, are nearly twice as long.

In view of the marked characteristics exhibited by this fungus, the writer considers it to be new to science and designates it as *Elaphomyces appalachiensis*.

Elaphomyces appalachiensis Linder sp. nov.

Fructificationes depresso-globosae, usque 6 x 5 mm. metientes; peridio externo "Prussian Red," hyphis 3–5 μ diametro, purpureis, laxe intertextis constante; peridio medio purpureo, hyphis subparallelis compactis plusminusve induratis et in funiculis intertextis, 120–140 μ crasso; peridio interno 300–500 μ crasso, "Light Cinnamon Buff," cellularum cum parietibus tenuibus composito, cellulis magnitudine variabilibus, bullatis vel pressu mutuo angulatis; gleba laxe gossypina, "Pearl Gray" siccata vel "Castor Gray" humecta; sporis sphaericis (6.5)-7.5–9 μ diametro, subhyalinis vel flavo-brunneis, subreticulatis vel reticulato-echinulatis.

Fructifications small, up to 6 x 5 mm., depressed globose, externally "Prussian Red." The peridium differentiated into three layers;—the outer peridium felty and composed of loosely interwoven hyphae which are 3–5 μ in diameter and purplish in color, derived from the middle peridium of purplish hyphae compacted in parallel strands which are closely interwoven to form a corneous prosenchymatous tissue 120–140 μ thick; and the inner peridium, "Light Pinkish Cinnamon" composed of thin-walled bullate or angular to loosely aggregated pseudoparenchymatous cells which are dilute brown. The gleba or "capillitium" is white in the center to "Pearl Gray" when dry or "Castor Gray" when wet. The ascospores are bluish in mass, appearing hyaline or dilute yellowish brown under the microscope, globose, (6.5)–7.5–9 μ in diameter, subreticulate to reticulate-echinulate.

Cades Cove, Great Smoky Mountain National Park, Tennessee, August 18, 1938, *A. H. Smith, 10,334*, parasitized by *Cordyceps intermedia* Imai. TYPE in the Farlow Herbarium of Harvard University and in the herbarium of the University of Michigan.

THE IDENTITY OF CERTAIN SPECIES OF THE SAPROLEGNIACEAE PARASITIC TO FISH[1, 2]

By WESLEY N. TIFFNEY[3]

Although fungi parasitic to fish have been recognized since 1777 (cf. the excellent review by Ramsbottom, 29) and during the last 75 years there have been frequent reports from many parts of the world of such parasitic Saprolegniaceae, the exact identification of many of the species listed is doubtful. This is particularly true of such reports as that of Huxley (18) wherein the organism observed as a parasite of salmon was stated to be either *Saprolegnia torulosa* or *S. ferax*, or that of Clinton (6) who identified his fungus as either *S. mixta* or *S. ferax*. There have been many other cases of doubtful identification. In some reports not only were adequate descriptions and illustrations of the fungi lacking, but also the identifications, when made, were apparently without adequate basis, since in some instances, for example, the authors recorded the observation of zoösporangia and chlamydospores only and yet identified the fungi in question as species typically forming sexual organs on which their specific identity was based. More important yet, many authors have failed to recognize the need for single spore isolations to eliminate chance contaminants and saprophytes, as well as the need for inoculation experiments to verify the pathogenicity of the fungi they have reported as parasites. It is well known that a variety of Saprolegniaceous fungi may be isolated from almost any fresh water in nature, and the dead tissue around a fish's wound furnishes an admirable substratum for growth. Therefore in reporting a fungus as parasitic it is highly desirable to ascertain whether that fungus really does act as a parasite.

[1] Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University no. 158.

[2] A portion of a thesis presented to the Faculty of Harvard University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[3] The writer wishes to acknowledge his indebtedness to Professor William H. Weston, Jr., under whose direction this work was done, for his invaluable assistance, and to Doctor David H. Linder who gave many helpful suggestions during the course of the investigation. The writer is also indebted to many friends in the laboratory who aided him in collecting.

The taxonomy of these fungi is further confused by the fact that the range of variation of critical structures in some of the most common pathogens is not well understood, a situation which leads to the description of new species or varieties that upon more exact study are shown to be untenable.

The desirability of establishing with greater certainty the identity of the Saprolegniaceae parasitic to fish seems self-evident. The subject of parasitism is of considerable interest to biologists, and in addition these fungi are of considerable importance to fresh-water fisheries, for they are a constant possible source of difficulty in the artificial propagation of fish and under favorable conditions may even cause serious epidemics. Although the problem of parasitism in this group has received considerable attention, the difficulty of applying the work of others to the writer's findings is immediately apparent because of the uncertainty of identifications encountered. Therefore in connection with his investigations the writer found a study of the identity of the organisms involved to be of fundamental importance.

Since the object of this investigation was to study the taxonomy of the parasitic members of the Saprolegniaceae, it was desirable first, to collect from diseased animals as many strains of fungi as possible; second, to isolate single strains in pure cultures; third, to prove that these strains were capable of parasitizing animals; and finally, to study them taxonomically and compare them with the species described in the literature.

MATERIALS AND METHODS

Fungi were collected from the following sources: ponds and streams in and around greater Boston, South Weymouth, and Falmouth, Massachusetts, Guilford, Connecticut, and Rindge, New Hampshire, and aquaria in the laboratories of Harvard University, Cambridge, Massachusetts. Fungi were collected in the field during every month in the year when the water was not frozen, and several collections were made during the winter from fish in laboratory tanks supplied with running tap water. The hosts from which the fungi were collected belonged to three classes of vertebrates, Pisces (various fish), Amphibia (newts, young and adult frogs) and Reptilia (turtles).

The fungi were isolated only from diseased animals which were still living in order to avoid the inclusion of purely saprophytic strains. This precaution has not always been observed by students of parasitic fungi, an omission which has in some cases cast doubt on the validity of otherwise significant results. In the present study, considering the diverse

character of the hosts and the widely different environments in which they were found, as well as the three years over which collections were made, it seems justifiable to assume that the fungi collected comprise a fair representation of the parasitic members of the Saprolegniaceae in this region.

All strains were obtained in culture by transferring a tuft of fungus from the living animal to sterile water containing sterile hemp seed, for although parasitic the fungi grew readily as saprophytes on such substrata. The fungi in these gross cultures were then isolated from other filamentous fungi and freed from bacteria as much as possible by means of repeated washings and transfers in sterile water. The resultant subcultures, which were maintained on hemp seed in sterilized water, were apparently normal and vigorous and in no way different from those found in nature. In this way 128 isolations of pathogenic members of the Saprolegniaceae were made from diseased aquatic animals in southern New England.

A preliminary study showed that the 128 strains of parasitic fungi isolated belonged to three genera of the Saprolegniaceae, *Saprolegnia*, *Achlya*, and *Dictyuchus*. The genus *Saprolegnia* contained by far the greatest number for it included 124 of the strains collected; of these 122 lacked sexual organs and therefore might be assigned to *S. parasitica* Coker, while the other two had sexual organs whose characteristics placed them in the species *S. ferax* (Gruith.) Thuret. *Achlya* was represented by 3 strains, 2 of which were identified as *A. flagellata* Coker, while the third, which lacked sexual reproduction, was called for the time simply *Achlya* sp. The one member of *Dictyuchus* which was isolated also lacked sexual organs and was designated temporarily *Dictyuchus* sp.

The figures above show the remarkable frequency with which *Saprolegnia parasitica* was encountered. The data obtained in the course of these collections indicate that it is by far the most common and consequently the most serious Saprolegniaceous parasite of aquatic animals in this region, hence intensive study of this species seemed especially necessary. The 122 strains of *S. parasitica* were carefully studied and compared and it was found that 66 of them agreed with Coker's type species in every detail. The other 56 isolations were found to differ in various respects although they still might be considered to be within the concept of the species. Comparison of these 56 strains showed that they could be divided according to their morphological characteristics into 7 groups, each containing several strains. Since

the strains in any one group were apparently identical in every respect, an intensive study of them all seemed unnecessary, therefore one strain from each group was chosen for further study and material of each of the other strains was preserved for reference.

The strains to receive further study were immediately isolated in pure culture. This involved eliminating the ever-present bacteria and was accomplished by two methods, the first that of repeated washing and transfer as adapted from the methods of Klebs (23), Kauffman (21), Horn (16), and Pieters (27); the second that of ultra-violet irradiation of culture media as described by Blank and Tiffney (3). The second method was employed almost exclusively in the latter part of the work. After the fungi had been freed from bacteria, single spore isolations were made by means of the technique of repeated washing and spraying outlined by Weston (36). The strains derived from single spores were kept in stock cultures.

Since this work was an investigation of the parasitic members of the Saprolegniaceae, especial care was taken to verify the pathogenicity of the strains investigated by following the classic principles of Koch. The fungi had been obtained from living animals with the characteristic symptoms of fish mold disease and had been isolated in pure culture, thus fulfilling the first two postulates of Koch. Each strain of fungus was then used to inoculate healthy fish under controlled conditions and was found to parasitize the fish, producing the typical symptoms of fish mold infection and in many cases death.[4] Each strain was reisolated from the diseased fish and obtained in pure culture and its identity with the strain which had been used in inoculation was then verified, thus fulfilling the remaining postulates of Koch. By following this procedure the writer was able to eliminate any doubt about the pathogenicity of the strains which were the subjects of the taxonomic studies which follow.

For the purpose of comparative studies the fungi were grown at room temperature on hemp seed in sterilized pond water since it was found that this method of culture produced abundant and typical structures similar to those found in nature. Morphological studies were made on large amounts of representative living material derived from single spores. Care was taken to avoid the inclusion of atypical structures due to poor or congested conditions of growth, excessive age of cultures, and similar unfavorable factors. Comparative studies were made both of the qualitative morphologic features and of the sizes of vegetative

[4] A detailed description of the technique used in inoculating fish will be given in a future paper dealing with the host range of *Saprolegnia parasitica*.

hyphae, zoösporangia, zoöspores, chlamydospores, oögonia, oöspores, and antheridia, as well as the presence and relative abundance of these structures. Since genera in this family are distinguished on the nonsexual apparatus and species primarily on the sexual organs, all the data from these comparative studies were none too adequate for identifying these fungi by means of such authoritative monographs as Humphrey (17), Fischer (12), von Minden (25), Coker (7), Nagai (26) and Lund (24).

COMPARATIVE STUDIES

1. *Saprolegnia*

Saprolegnia is by far the most important genus of the filamentous fungi capable of parasitizing aquatic animals. In the literature 5 species of this genus, *S. ferax*, *S. torulosa*, *S. monoica*, *S. mixta* and *S. parasitica* (reported also as *Isoachlya parasitica*), and one variety, *Saprolegnia parasitica* var. *Kochhari*, have been reported as parasitic. *Saprolegnia ferax* and *S. parasitica*, both of which have been reported many times, are the important pathogens of the group, while the other three species are considerably less important since each has been reported only once. In fact, the inclusion of *S. torulosa* de Bary as a parasite may be questioned, for Huxley (18), who reported it, stated that the organism which he observed on salmon was either *S. torulosa* or *S. ferax*. The same is true of *S. mixta* de Bary for Clinton (6), author of the sole report, identified his parasitic fungus as either *S. mixta* or *S. ferax*. *Saprolegnia monoica* Pringsheim was reported once as a parasite, by Walentowicz (35), who described an epidemic among carp in Galicia, Poland, and stated that *S. monoica* was found on the fish in conjunction with *Achlya Nowickii*.

Saprolegnia ferax (Gruith.) Thuret is a recognized pathogen for it has been reported as parasitic by at least nine authors: Agersborg (1), Smith (32), Clinton (6), Hardy (13), Huxley (18), Stirling (33), Blanc (2), Schnetzler (31), and Collins (9). By the present writer two strains of this species were isolated, one from *Chelydra serpentina* and one from *Necturus maculosus*. Both were proved to be pathogenic by inoculation experiments and both formed sexual organs when grown on hemp seed in water cultures, bearing the typical oögonia and oöspores of the species. The identity of these two strains seems clear. Since *S. ferax* is a definite species which has been well described by Coker (7), Lund (24), and others it is not necessary to describe it here.

It is of interest to note, however, that the form obtained from *Necturus*

did not reveal its identity for a considerable period after its isolation. During this period it was tentatively assigned to *S. parasitica* because of a very close resemblance to that species, particularly in the number and appearance of the chlamydospores and the diameter of the cystospores (the encysted zoöspores). In *S. parasitica* Coker the cystospores are described as ranging from 9 μ to 13 μ with an average diameter of 10 μ, while in the form under consideration the cystospores ranged from 9 μ to 14 μ, with the greatest number having a diameter of 11 μ. However in regard to the type of zoösporangial renewal there was a marked discrepancy since in the writer's form renewal was accomplished usually by proliferation through the old sporangium and only occasionally by cymose branching, while in *S. parasitica* cymose branching was the predominant method. Although at first this difference might appear to be an important one the method of zoösporangial renewal will be shown to be of questionable diagnostic value in *S. parasitica* and hence not to be given too great significance here. When the form from *Necturus* was finally induced to fruit by growing it on a whole hemp seed with the seed coat punctured by only one small pin prick, a method used successfully by other authors, the characteristics of its sexual organs showed it to be *S. ferax*.

Saprolegnia parasitica Coker is probably the most common Saprolegniaceous parasite of aquatic animals. The species was established by Coker in 1923 to include the frequently encountered parasitic members of the genus, which, lacking sexual organs, could not correctly be identified with any of the species then described. The new species was distinguished chiefly by the facts that it was a parasite, that it did not form sexual organs but reproduced solely by asexual means, and that it often accomplished zoösporangial renewal by cymose branching as well as by proliferation through the old sporangium. Since its description the species has been collected by many authors. In the writer's isolations *S. parasitica* was the form most frequently secured, since 122 of the 128 strains isolated were identified as this species. From the remarkable predominance of this species it is reasonable to suppose that the sterile parasitic strains frequently mentioned in the literature and ascribed to various sexual species may have been the form now known as *S. parasitica*. Furthermore the occurrence of this species over an extremely wide geographical range, as indicated by the reports from North Carolina by Coker (7), from Bohemia by Cejp (4), from Japan by Nagai (26), from Denmark by Lund (24), and from India by Chaudhuri and Kochhar (5), as well as the remarkable frequency with which it

has been shown to occur in a restricted locality (the writer's collections in southern New England), indicates its prevalence and ubiquity. Its economic importance and the reason for the volume of literature on the subject are apparent if one adds that the fungus appears on a wide variety of hosts during every month of the year and under favorable conditions may cause destructive epidemics.

Saprolegnia parasitica was isolated by the writer from members of three vertebrate classes, Pisces, Amphibia, and Reptilia. The following species were found as hosts:[5] *Fundulus heteroclitus, Crassius auratus, Eupomotis gibbosus, Perca flavescens, Ameiurus* sp., *Erimyzon* sp., *Necturus maculosus, Triturus viridescens, Rana pipiens*, and *Chrysemys concinna*. The fungi were collected throughout the year under a variety of natural and artificial conditions. As has been stated previously the 122 strains collected were cultured, purified and divided into 8 groups, each containing a number of apparently identical strains; one strain from each group was chosen as its representative. (The first group was represented by a subculture of the type culture of *S. parasitica* Coker which was deposited by Coker in the Centraalbureau voor Schimmelcultures, Baarn, Holland.) These 8 representative strains showed distinct differences even upon superficial examination although they all possessed the characteristics of sterility and pathogenicity typical of *S. parasitica*. The differences they exhibited, however, emphasized the desirability of comparative studies to determine the range of variation possible within the species.

Since sexual organs were lacking in these strains it was necessary to resort to studies of their asexual reproductive structures and vegetative growth. Comparisons were made of the qualitative morphological features and sizes of the vegetative hyphae, zoösporangia, zoöspores, and chlamydospores. In general the strains showed no essential variations in qualitative characteristics and it was necessary to make measurements of various structures in order to demonstrate their differences. The results of these measurements, which are given in Table I, are in every case based on at least 100 observations and are presented in the form "11–22–38" in which the first and last numbers represent the extremes observed and the middle number represents the measurement obtained most frequently. These figures represent random selection from a large quantity of living material grown and studied under similar conditions, as described previously. The total diameter of growth was

[5] Teleostei determined from Jordan and Evermann (19); Amphibia and Reptilia from Pratt (28), and Ditmars (11).

determined by measuring the mature seven-day colonies grown on hemp seed in water. To determine comparative sizes of zoöspores the cystospore stages were measured because they were stationary and spherical, whereas the primary and secondary swarming stages were motile and comparatively irregular in shape. Such features as the method of zoösporangial renewal and the shape and arrangement of chlamydospores were determined by the examination of large amounts of representative material.

The significant results of these studies on the 7 representative strains of *S. parasitica* isolated by the writer and on a subculture of Coker's type culture of the species, for comparison, are presented in Table I. The writer wishes to emphasize the fact that these measurements are not in any sense intended as an exact quantitative analysis of the features involved, but merely as a convenient and definite way of comparing their sizes.

The vegetative hyphae of all strains were essentially alike in qualitative characteristics, but they did exhibit some differences in size as the measurements of the first three characters listed in Table I will show. The total diameter of colony growth on hemp seed (which is of course an expression of hyphal length) varied slightly in the different strains but the range of variation was so small that it seemed to have no significance for the purposes of this study. Considerably greater variation was found in the diameter of the hyphae at the base and at the tip, the dimensions being twice as large in some strains as in others. Another feature which is not listed in the table but which was evident upon observation of the fungi was the tapering shape characteristic of the hyphae of some strains. Strain 3, in which the hyphae were most frequently 40 μ in diameter at the base and 9 μ in diameter at the tip, had the most conspicuously tapering hyphae, while Strain 1 (Coker's type culture) and Strain 11, in both of which the diameter of the base of a hypha was in general only 4 μ greater than the diameter of its tip, had hyphae that did not taper, and the other 5 strains formed a transitional series between these two extremes.

The differences in vegetative growth discussed above have no apparent diagnostic significance, however, for a survey shows that although the fungi can be arranged in a series on the basis of any one of the above characters, the arrangement is different for each character and there is no evident correlation between them. It would seem therefore that the slight differences exhibited in the vegetative growth of these strains have no diagnostic value.

TABLE I

Comparative Morphological Studies of Strains of Saprolegnia parasitica

CHARACTER	STRAIN 1, SUBCULTURE OF THE TYPE	STRAIN 3	STRAIN 4	STRAIN 6	STRAIN 8	STRAIN 11	STRAIN 12	STRAIN 16
7 day colony on hemp seed, diameter in mm.	9–12–16	9–12–16	5–13–18	9–13.5–21	9–10–13	7–9–11	10–12–15	9–12–16
Vegetative hyphae, diameter in μ								
At base	12–21–29	23–40–55	15–23–33	25–44–54	18–36–59	18–25–36	25–44–54	14–28–48
At tip	11–17–30	6–9–12	10–16–23	15–26–46	15–24–30	11–21–34	15–28–46	7–10–15
Zoösporangia								
Length in μ	135–260–469	70–108–160	118–205–262	250–465–775	155–231–440	95–185–338	247–407–893	98–160–220
Width in μ	13–21–38	6–11–14	9–12–26	11–24–49	20–34–45	19–25–30	26–36–53	11–18–27
Renewal by proliferation thru old sporangia	0–20%	70%–100%	0–20%	0–20%	0–30%	0–20%	40%–60%	85%–100%
Renewal by cymose branching	80%–100%	0–30%	80%–100%	80%–100%	70%–100%	80%–100%	40%–60%	0–15%
Cystospores, diameter in μ	9–10–13	8–10–11	9–10–12	9–11–12	9–10–12	8–10–13	9–11–14	6–8–10
Chlamydospores								
Abundance	Abundant	Abundant	Sparse	Abundant	Abundant	Abundant	Moderately abundant	Sparse
Position	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, or intercalary	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in cymosely branching chains	Terminal, intercalary, or in chains
Shape	Globose to clavate	Globose to clavate	Globose, rarely clavate	Globose to clavate	Globose to clavate	Globose to clavate	Globose to clavate	Globose to clavate

One would expect to find more definite and constant characteristics upon studying the zoösporangia, especially since the methods of formation and discharge of these structures are of considerable importance in the classification of the Saprolegniaceae. In the species under consideration Chaudhuri and Kochhar (5) have made a difference in zoösporangial length the distinguishing characteristic of a new variety, *S. parasitica* var. *Kochhari*, while Nagai (26) has even transferred *S. parasitica* to another genus, *Isoachlya*, because of one of its methods of zoösporangial renewal. A comparative study of the characteristics of the zoösporangia of Coker's type culture of the species and the 7 strains collected by the writer would therefore seem to be of considerable interest.

No significant differences in the shapes of the zoösporangia of the 8 strains were noted. They all exhibited some irregularity in shape at times, a feature of this species which has been observed by Coker (7), Lund (24), and others, but there was nothing which would serve to distinguish one strain from another.

However, the 8 strains did display marked differences in the sizes of zoösporangia, and the results of measurements of their lengths and widths are presented in the table. The zoösporangia of the 8 strains ranged in length from a minimum of 70 μ in Strain 3 to a maximum of 893 μ in Strain 12. This is a maximum range greater than any that has yet been set for this species. However, if the 8 strains are arranged according to their sporangial lengths as shown below it will be evident that the measurements overlap markedly and form a continuous series with no conspicuous gaps.

S PARASITICA—STRAIN NUMBER	LENGTHS OF ZOÖSPORANGIA IN μ		
	Minimum	Most frequent	Maximum
3	70	108	160
16	98	160	220
11	95	185	338
4	118	205	262
8	155	231	440
1 (Coker's type)	135	260	469
12	247	407	893
6	250	465	775

If an investigator were to collect a culture like Strain 3 and another like Strain 12 without seeing the intervening types it would be quite

possible for him to assume that he had two different varieties of the same species. On the other hand, a series such as the one above in which the measurements overlap as they do presents an entirely different picture. The writer believes himself justified in assuming that he is dealing with one species whose range of variation upon intensive study has appeared to be much greater than the original descriptions would lead him to believe. It is his contention that this range of variation is not a sufficient basis for the establishment of new varieties, but rather should be considered as an extension of the present range of variation within the species.

In 1935 Chaudhuri and Kochhar (5) in a survey of the water molds of India established a new variety, *S. parasitica* var. *Kochhari* Chaudhuri, on the basis of zoösporangial length. These authors described the length of *S. parasitica* Coker as ranging between 280 μ and 560 μ, while the length of the new variety of Chaudhuri was stated to be 400 μ to 600 μ, a range much more restricted than any the present writer has found in the forms he has studied. According to the evidence presented above the range of zoösporangial length in *S. parasitica* should be extended to include forms ranging from 70 μ to 893 μ. Consequently it is the writer's opinion that the variety *S. parasitica* var. *Kochhari* Chaudhuri has been established on an inadequate basis.

Zoösporangial width was found to vary in proportion to length, for the width was in general from 5% to 15% of the length. Therefore the arrangement of strains as presented on zoösporangial length would also hold to an equal degree for zoösporangial width.

The method of zoösporangial renewal, whether by growth through the old sporangium or by cymose branching from below the base of the old sporangium, is often considered an important feature in the classification of Saprolegniaceous fungi. One of the distinguishing characteristics of the species *S. parasitica* is that it shows cymose branching, "very often proliferating from the side below as in *Achlya*" (Coker, 7, p. 58). Kauffman (22) gave even greater weight to this character, for in 1921 he made it the chief basis for the formation of the genus *Isoachlya*, saying, "The genus is characterized and distinguished, in the main, by the presence of the cymose or *Achlya* mode of formation of secondary sporangia, coupled with diplanetic zoöspores" (Kauffman, 22, p. 231). Coker recognized the genus *Isoachlya* but nevertheless did not place *Saprolegnia parasitica* in it when he described this species two years later. Subsequently Nagai (26) and Lund (24) both pointed out that according to the definitions of the genus and the species, *S. parasitica*

Coker should be placed in *Isoachlya* and Nagai made this change, calling the organism *I. parasitica* (Coker) Nagai. Coker (8), however, in the *North American Flora* still retains the species in the genus *Saprolegnia*.

In the course of the writer's studies, however, this character proved to be of less diagnostic value than might be expected, as an examination of the data presented in Table I will show. In Coker's type culture (Strain 1) and Strains 4, 6, and 11 zoösporangial renewal was generally accomplished (in 80% to approximately 100% of the cases) by cymose branching and rarely (in 20% to almost none of the cases) by growth through the empty sporangium. In Strain 8 cymose branching was somewhat less frequent, including approximately 70% of the cases. In Strain 12 renewal was effected about equally by the two methods. In Strain 3 only about 30% of the renewal was accomplished by cymose branching, while growth through the old sporangium was the predominant method. In Strain 16 cymose branching was rare and renewal was accomplished almost wholly (85% to almost 100%) by proliferation through the old sporangium. It will be seen that the 8 strains formed a transitional series from one extreme, in which renewal was effected almost completely by cymose branching, to the other, in which it was accomplished almost completely by growth through the old sporangium, with intergrading forms between. As has been stated before, the writer found no valid reason for excluding any of the strains studied from the species *S. parasitica;* consequently he believes that the data presented above indicate that the limits of this species should be extended to include those forms in which cymose branching is not necessarily the predominant mode of zoösporangial renewal.

In view of the facts presented above the writer sees no necessity for transferring *S. parasitica* to the genus *Isoachlya*. As these studies show, the *Achlya*-like cymose branching is not a conspicuous feature of all strains of the species. Moreover, cymose branching as one method of zoösporangial renewal is an accepted feature in several species of *Saprolegnia,* i.e., *S. ferax* (Gruith.) Thuret, *S. asterophora* de Bary, *S. spiralis* Cornu, *S. diclina* Humphrey, *S. anisospora* de Bary and others (Coker 7). To the writer, therefore, it seems preferable to retain *S. parasitica* in its original genus.

Furthermore, it seems fitting to inquire at this point whether the method of renewal of zoösporangia, which may vary to a marked degree within the confines of one species, is a valid character upon which to base generic distinctions. An exact study of several members of the genera involved is highly desirable and should do much to clarify the situation,

for it seems probable that greater significance has been attributed to the mode of zoösporangial renewal in certain forms than the present knowledge of this character and the amount of exact study devoted to it would warrant.

In the method of discharge of zoöspores no significant differences between the 8 strains were noted. Occasional irregularities occurred, particularly when conditions had become somewhat unfavorable, but this is a recognized occurrence and has no importance for the purposes of this study. Upon discharge the spores swarmed about, at length coming to rest as spherical cystospores, from which the secondary spores later escaped and swam about in the manner typical of the genus *Saprolegnia*. In a few instances spores were observed to go through repeated swarming periods. This phenomenon has been observed by other writers (Weston, 36, Höhnk, 15).

Comparative sizes of the zoöspores were determined by measuring the cystospores, since they were stationary and spherical and therefore could be measured with more accuracy than the active swimming spores. Little variation was shown in cystospore size, as may be seen from Table I. The cystospores of 7 of the strains closely approximated one another, ranging from 8 μ to 14 μ in diameter, being most frequently 10 to 11 μ. Only Strain 16 showed some difference, having cystospores that ranged from 6 μ to 10 μ in diameter, being most frequently 8 μ.

Chlamydospores or resting bodies, while one of the most noticeable features of these fungi in culture, were disappointing as sources of diagnostic characters. Some slight differences between the various strains were observed in the abundance and position of these bodies. They were sparse in Strains 4 and 16, moderately abundant in Strain 12 and abundant in the 5 other strains. In addition, Strains 4 and 16 lacked the cymosely branching chains of chlamydospores that were present occasionally in Strain 12 and frequently in the other strains. These differences, while interesting, are hardly marked enough to justify much significance being given them, especially since they show no correlation with the various characteristics previously described.

None of the strains formed sexual organs under any conditions, consequently comparative studies were limited to the asexual features discussed above.

It is interesting to note that Kanouse (20) has reported a case which seems to be parallel with the findings of the writer in some respects. Her organism, isolated from fish eggs and identified as *S. parasitica*, although typically asexual did respond to certain salt solutions and

produced fruiting bodies. One of the writer's forms, which was isolated from *Necturus* and appeared to be *Saprolegnia parasitica* failed to fruit in the same salt solutions described by Kanouse, but did fruit finally when grown on hemp seed, and was identified as *S. ferax*. Both forms in their asexual stages agreed with Coker's description of *S. parasitica* and both upon fruiting exhibited the characteristics of two entirely different species. It seems to the writer that these cases are indicative of the possibility of a curious situation in *S. parasitica*, namely, that this "species," defined on asexual characters, may in reality be composed of forms of heterogeneous origin which, if they could be induced to fruit, might be found to belong to several different species within the genus *Saprolegnia*.

Since the individual significance of each of the writer's findings has been discussed in the foregoing pages there is no need for repetition here. Taken as a whole these studies lead to the conclusion that while *Saprolegnia parasitica* shows considerable variation in its characteristics there is nevertheless no valid ground for dividing it into more than one species, and therefore the writer believes that for the present these findings should be interpreted only as extending the range of variation possible within the species. The rather unsatisfactory nature of this conclusion illustrates clearly the difficulty of defining a species on asexual characters alone.

The results of these studies lead one to speculate on the nature of the species *S. parasitica*. It may be that, like certain members of *Dictyuchus* studied by Couch (10), these sterile strains of *Saprolegnia* are in reality segregated unisexual strains which when combined with complementary strains should result in sexual reproduction. On the other hand, it is possible that these fungi, which do not reproduce sexually under the usual cultural conditions, may be induced to fruit when the proper combination of environmental factors is found. Finally, if neither of these suppositions can be dèmonstrated we shall be forced for the present to conclude that the organism is a species which under ordinary conditions has lost the power of sexual reproduction. For the present it seems best to consider *Saprolegnia parasitica* a species of convenience, and it should be treated as such until further studies have demonstrated its exact nature.

2. *Achlya*

The genus *Achlya* contains four species which have been reported as parasitic, the identity of which is unquestioned since in every case the

fungus formed sexual organs which permitted accurate identification. *Achlya prolifera* (Nees) de Bary appears most often in the literature, for it has been reported by Robin (30), Schnetzler (31), and Blanc (2). *Achlya racemosa* Hildebrand was observed as a parasite by Humphrey (17) and by Hine (14) who also reported *A. polyandra* Hildebrand. It might be noted that none of these three species was observed on more than a few individual hosts, and none was demonstrated to be pathogenic by inoculation experiments.

Achlya flagellata Coker was found as a parasite by Tiffney and Wolf (34) in Massachusetts and Wisconsin, respectively. Wolf observed a serious epidemic among trout in a Wisconsin fish hatchery; *A. flagellata* was found to be the cause of the epidemic and no other fungi were involved. The present writer reported two isolations of this species from diseased animals, one from *Triturus viridescens* on which the fungus occurred in conjunction with *Saprolegnia parasitica*, and one from *Lebistes reticulatus*. The second strain was used to inoculate *Fundulus heteroclitus* and was shown to act as a wound parasite.

In addition, the writer succeeded in isolating a sterile strain of *Achlya* from the outer portion of the shell of *Chelydra serpentina* where it was associated with *Saprolegnia ferax*. Inoculation experiments showed this strain, like the *A. flagellata* mentioned above, to be capable of a parasitic existence only if the host's resistance had been lowered by severe injury, a fact which places it in the category of wound parasites. This sterile *Achlya* was maintained in culture for approximately a year and a half, and during that time was subjected to a variety of environmental conditions but could not be induced to fruit. Since this organism, being non-sexual, cannot with certainty be referred to a recognized species it seems desirable to include a brief description of it here.

Achlya sp.

Growth comparatively slow, requiring 7 days or more to reach its greatest extent; mature colony 5–15 mm. in diameter on hemp seed; hyphae 30–70 μ in diameter at the base, tapering to 20–27 μ at the tip; zoösporangia numerous, 200–420 μ in length and renewed by repeated cymose branching; cystospores 10–12 μ in diameter; chlamydospores spherical to clavate, single or in chains, occasionally reaching a diameter of 100 μ or more.

This genus has not, heretofore, been considered particularly important as a parasite of aquatic animals, but it seems probable, especially in view of the serious epidemic reported by Wolf (Tiffney and Wolf, 34), that future observations may disclose the genus to be more important in this respect than has been supposed.

3. *Dictyuchus*

The genus *Dictyuchus*, as far as the writer is aware, contains no species which have been reported as parasitic to animals.

One form belonging to this genus was isolated by the writer from a living *Triturus viridescens*. Inoculation experiments showed the fungus to be a parasite, but like the species of *Achlya* previously described it could be classed only as a wound parasite. This organism never formed sexual fruiting bodies although it was subjected to a variety of cultural conditions over a period of ten months. In view of the findings of Couch (10) it seems probable that this form is a member of the species *Dictyuchus monosporus* Leitgeb.

From the foregoing report it is apparent that the writer found *Saprolegnia* to be far the most important genus of the fungi parasitic to fish, since it was so frequently isolated. *Achlya* was found to be considerably less important and *Dictyuchus* of no real importance in this respect. This survey, by reason of the time devoted to it and the area covered, probably gives a reliable index to the situation in New England. It would be most interesting to have similar surveys from other regions for purposes of comparison.

SUMMARY

128 strains of Saprolegniaceae were isolated in southern New England from living diseased aquatic animals.

The representative strains of fungi which received intensive study were isolated in pure culture as single spore strains and proved to be pathogenic by inoculation experiments.

2 strains of *Saprolegnia ferax* were obtained. One of them, which in its asexual stage closely resembled *S. parasitica*, was induced to fruit only by special cultural methods.

122 strains of *Saprolegnia parasitica* were collected. Comparative studies of a subculture of Coker's type culture of this species and representative strains isolated by the writer showed that the more or less marked variations displayed in vegetative and asexual reproductive structures should not be considered to be of diagnostic value, but should serve only to extend the range of variation possible within the species. In the writer's opinion *S. parasitica* should be considered a species of convenience until further study has demonstrated its exact nature.

The variety *S. parasitica* var. *Kochhari* is considered to have been established on an inadequate basis, since the characteristic upon which

it was based is included in the range of the species as it is interpreted here.

The character "renewal of zoösporangia by cymose branching" was shown to be more variable and correspondingly less significant than has been thought; consequently the writer prefers not to accept the transfer of *Saprolegnia parasitica* to the genus *Isoachlya,* but to retain it in its original genus.

2 strains of *Achlya flagellata,* 1 *Achlya* sp., and 1 *Dictyuchus* sp. were isolated and shown to be wound parasites. This is, to the writer's knowledge, the first observation of a member of *Dictyuchus* parasitic to animals.

AMERICAN INTERNATIONAL COLLEGE,
SPRINGFIELD, MASSACHUSETTS.

LITERATURE CITED

1. AGERSBORG, H. P. K., Salient problems in the artificial rearing of salmonoid fishes, with special reference to intestinal fungisitosis and the cause of white-spot disease. Trans. Am. Fish Soc. **63**: 240-250. 1933.
2. BLANC, Notice sur une Mortalité exceptionelle des brochets. Bull. Soc. Vand. Nat. **23**: 33-37. 1887.
3. BLANK, I. H., AND TIFFNEY, W. N., The use of ultra-violet irradiated culture media for securing bacteria-free cultures of *Saprolegnia.* Mycologia **28**: 324-329. 1936.
4. CEJP, K., Contributions to the knowledge of Saprolegniales of Bohemia. II. Bull. Acad. Sci. Bohème, p. 13, 1934.
5. CHAUDHURI, H., AND KOCHHAR, P. L., Indian water molds. I. Proc. of the Indian Acad. of Sci. **2**: 137-154. 1935.
6. CLINTON, G. P., Observations on a fungus (*Saprolegnia*) infesting fish Bull. U. S. Fish Com. **13**: 163-172. 1894.
7. COKER, W. C., The Saprolegniaceae. Univ. of N. C. Press, Chapel Hill, N. C. 1923.
8. ——— AND MATTHEWS, V., North American Flora, Vol. 2, Part 1. New York Botanical Garden. 1937.
9. COLLINS, M. I., Note on certain variations of the sporocyst in a species of *Saprolegnia.* Proc. Linn. Soc. N. S. W. **45**: 277. 1920.
10. COUCH, J. N., Heterothallism in *Dictyuchus,* a genus of the water-moulds. Ann. Bot. **40**: 849-881. 1926.
11. DITMARS, R. L., The Reptile Book. Doubleday Page & Co. New York. 1907.
12. FISCHER, A., Saprolegninae. Rabenhorst's Krypt. Fl. 1, Ab. **4**: 310, Leipzig, 1892. (2nd edition.)
13. HARDY, A. D., Association of alga and fungus in salmon disease (*Myxonema* and *Saprolegnia*). Proc. Roy. Soc. Vict. N. S. **23**: 27. 1911.
14. HINE, F. B., Observations on several forms of *Saprolegnia.* Am. Quart. Mic. Jour. **1**: 18-24. 1878-79.

15. HÖHNK, W., Polyplanetism and zoöspore germination in Saprolegniaceae and *Pythium*. Am. Jour. Bot. **20**: 45–62. 1933.
16. HORN, L., Experimentelle Entwickelungsanderungen bei *Achlya polyandra* de Bary. Ann. Myc. **2**: 205. 1904.
17. HUMPHREY, J. E., The Saprolegniaceae of the United States, with notes on other species. Trans. Am. Phil. Soc. **17**: 63. 1893.
18. HUXLEY, T. H., *Saprolegnia* in relation to salmon disease. Quart. Jour. Mic. Soc. **22**: 311. 1882.
19. JORDAN, D. S., AND EVERMANN, B. W., Fishes of North and Middle America. Proc. U. S. Nat. Mus. 4 Vol., 1896.
20. KANOUSE, B. B., A physiological and morphological study of *Saprolegnia parasitica*. Mycologia **24**: 431–452. 1932.
21. KAUFFMAN, C. H., A contribution to the physiology of the Saprolegniaceae, with special reference to the variations of the sexual organs. Ann. Bot. **22**: 361–387. 1908.
22. ———, *Isoachlya*, a new genus of the Saprolegniaceae. Am. Jour. Bot. **8**: 231–237. 1921.
23. KLEBS, G., Zur Physiologie der Fortpflanzung einiger Pilze. 2. Jahrb. f. wiss. Bot. **33**: 513. 1899.
24. LUND, A., Studies on Danish freshwater Phycomycetes. Mem. Acad. Roy. Sci. Danemark, ser. 9, **6**, No. 1. Copenhagen, 1934.
25. MINDEN, VON, Saprolegniineae. Kryptogamen Flora Mark Brandenburg **5**: 479. 1915.
26. NAGAI, M., Studies on the Japanese Saprolegniaceae. Jour. Facul. Agr. Hokkaido Imp. Univ. Sapporo **32**: 1–43. 1931.
27. PIETERS, A. J., The ferax group of the genus *Saprolegnia*. Mycologia **7**: 307–314. 1915.
28. PRATT, H. S., A Manual of Land and Fresh-water Vertebrate Animals of the U. S. P. Blakiston Son and Co., Philadelphia, 1923.
29. RAMSBOTTOM, J., Some notes on the history of the classification of the Phycomycetes. Trans. Brit. Myc. Soc. **5**: 324. 1916.
30. ROBIN, C., Histoire naturelle des Vegetaux parasites, etc., qui croissent sur l'Homme et sur les Animaux vivants. B. Bailliere. Paris, 1853.
31. SCHNETZLER, J. B., Infection d'un Larve de Grenouille par *Saprolegnia ferax*. Archiv. de Sci. Phys. et Nat. Genève **18**: 492. 1887.
32. SMITH, W. G., The salmon disease (*S. ferax*). The Gardener's Chron. **9**: 560–561. 1878.
33. STIRLING, A. B., Additional observations on the fungus disease affecting salmon and other fish. Proc. Roy. Soc. Edinburgh **10**: 232–370. 1880.
34. TIFFNEY, W. N., AND WOLF, F. T., *Achlya flagellata* as a fish parasite. Journ. Elisha Mitchell Sci. Soc. **53**: 298. 1937.
35. WALENTOWICZ, Karpfenfest in Kaniow. Oesterreich vierteljahresshrift wiss. veterinärkunde. Bd. 64. 1885.
36. WESTON, W. H., Repeated zoöspore emergence in *Dictyuchus*. Bot. Gaz. **68**: 287–296. 1919.

A REMARKABLE SAPROPHYTIC FUNGOID ALGA*

By W. C. Coker and Leland Shanor

Plates 22 and 23

In the spring of 1937 the senior author, while scouting in upper Chatham County, about 7½ miles from Chapel Hill, North Carolina, noticed a white fluffy fungoid-looking growth in a small spring stream near the base of a large sawdust pile which had been burning for at least 4 months. This growth was attached to objects in the stream such as leaves, roots, sticks, and rocks, but more frequently to blades and panicles of grass which were bent over into the running water (Figs. 19, 20). Thinking that it was probably *Leptomitus*, one of the well known water molds that we have been observing for years, material attached to grass and twigs was brought into the laboratory for study. On examination this proved to be not a water mold but a remarkable plant that is the subject of this paper.

These first collections made were kept in the laboratory for a short time, but soon died from the increased bacterial contamination. Again in the fall of the same year, a visit to this location showed that the plant was still available, and on this same excursion a second station was found about two miles from the first, our plant growing again under similar circumstances. In the spring of 1938, the plant having been found growing abundantly in this latter stream, it was decided to study it more carefully. This work was started on April 23, 1938, and cultural studies begun on April 30.

If this plant had been green, we would have said at once that it was probably a *Stigeoclonium* or a close relative of that genus in the Chaetophoraceae. The size and whole organization of the plant body is remarkably similar to that of members of this group. Each individual plant body consists of a main axis, generally tapering upward and branching at rather regular intervals from the upper ends of the cells, these rebranching again several times and finally ending in long slender

* A preliminary report on this paper was presented before the National Academy of Sciences, meeting at Chapel Hill, North Carolina, October 25, 1938.

cells which taper to extremely fine points (figs. 1, 2, 4). The basal cell or cells give off numerous irregular and branching rhizoidal holdfasts (fig. 7, 8, 24), just as in *Stigeoclonium* and its relatives. The attachment of these plants seems to be entirely for support and not for nutrition, as is indicated by experiments cited below.

The cells of this plant appear perfectly transparent in transmitted light and there is no trace of a body that might be called a colorless chromatophore, if one could use such a contradictory term. The cytoplasmic lining of the large cells is confined to the cell walls and is extremely thin, in fact hardly observable with ordinary magnification, being usually less than 1.5μ thick except in the ends of the cells. In the young more narrow cells the protoplasm is not confined to the region of the cell walls but extends here and there across the interior giving a very vacuolated appearance. The nuclei in the largest cells are large and obvious, measuring from 5.6–8.6μ in diameter, and have a very conspicuous gleaming nucleolus (figs. 7, 25). Nuclei in small tip cells measure 1.7–2μ in diameter. In a cell of average size, when seen in surface view after being killed in the fumes of osmic acid and stained with gentian violet, the cytoplasm seems to be arranged in inconspicuously diagonal strands reminding one of the arrangement of the chloroplasts of *Nitella* (fig. 6). In large old plants a number of the basal cells and the rhizoidal holdfasts arising from them appear to be dead and empty. We have been unable to demonstrate a nucleus in the holdfast cells.

GROWTH IN NATURAL ENVIRONMENT AND CHEMICAL ANALYSIS OF THE BRANCH WATER

At our first station, the spring supplying the branch is located only about 20 yards above the upper end of the burning sawdust pile. The fluffy growth of our plant appeared at the uppermost point that seepage from the pile would likely enter, and above this line, which was a sharp one, it was entirely absent. From this point downward it was abundant for many yards. Perhaps 30 or 40 yards below this seepage, the branch disappeared in a thick tangle of *Smilax* and was not examined further.

At the second station, the spring was located in a very similar position in relation to the burning sawdust pile as it was at the first, but after about 50 yards, this small stream from the spring entered a larger branch and the growth of our plant in this stream is quite remarkable. Growth

in the smaller stream began just opposite the sawdust pile and continued down this and on into the larger one. Above the point in the large stream where the small stream entered there was no sign of any growth. Likewise, no trace of growth could be noted in the smaller stream above the seepage line. The larger stream here is filled with large rocks and these were abundantly covered with this fluffy growth, sometimes to a depth of as much as 5 cm. (fig. 19), in addition to the usual abundance of it found attached to roots, twigs, grass blades and panicles. Growth attached to grass has been observed in this stream as far as about ½ mile from the point where the smaller stream entered it.

Cool temperatures favor the growth of this plant, and we have been unable to find it in the warmer seasons. Most of the collections have been made either during the late fall and winter or during the spring months. Our last collection made during the summer of 1938 was on June 26. On September 15, no trace of growth could be found at either station and on other visits on September 27 and October 6, still no growth could be seen. On October 13, a few blades of grass with a small amount of growth attached were collected at our second station, but not until October 24 was there an abundance of material available.

The fact that no growth appeared above the seepage point in both of these stations indicates clearly that some nourishing substance finds its way into the water from these burning sawdust piles which is essential to the growth of the plant. Further evidence that the nourishment for growth is obtained from the water and not from the substratum is shown by the fact that growth on rocks in the stream below the seepage is equal to that of plants attached to organic objects. The attachment of these plants to objects in water is, then, evidently for support alone. Still further evidence that the nourishment comes from the burning sawdust is the following observation. Sometime during the past summer the sawdust pile at the first station quit burning, and when the growth reappeared late in October at the second station, none could be observed in the stream where it was first found. Although there has been an abundant growth in the stream at the second station all during the winter and spring months, the plant has not again reappeared at the first station.

Members of our chemistry department very generously undertook to analyse the branch water in which the plants were growing. We are greatly indebted to Dr. Edward Mack, Jr., Dr. R. W. Bost, and Dr. E. C. Markham for their most valuable assistance in this work. The

results of their analysis, so far as it was thought necessary to carry it, is as follows:

pH 598, not much more acid than distilled water.

	mg. per liter
Total solids (dried at 100 C.)	126
Total solids (after burning)	47
Chlorine (Cl)	5
Iron	1
Nitrogen (as free NH)	0.04
Nitrogen (albuminoid)	0.6
Nitrogen (total organic)	0.5

Results when one liter was evaporated to 25 cc. and tested for various possible organic substances:

1. $FeCl_3$ test was negative, showing no tannins or phenols.
2. Indophenol test negative, showing no phenols.
3. Bromine water, test negative, showing no unsaturates of phenols.
4. Schiff's test was positive, showing no aldehydes (sugars do not respond to this test).
5. Molisch test was positive, showing carbohydrates.
6. Quantitative determination of reducing sugars calculated as glucose, 6.9 mg. per liter.
7. Permanganate titration of reducing substances calculated as glucose, 6.0 mg. per liter.

This analysis showed that the branch water was almost free from mineral content but contained a noticeable amount of reducing sugar. It also has a very characteristic acid sweet odor.

REPRODUCTION AND DEVELOPMENT

To our surprise, as well as to theirs, the chemists found that after filtering the water brought in with the plants through a hard filter which would usually remove all visible particles, in a few days a fluffy growth was seen throughout the water which gave it a milky appearance. We have found that the ultimate delicate tips of the end branches of this plant are very easily thrown off, so that if handled at all these separated tips would always be found floating freely in the water. These tips may be single-celled or be made up of as many as seven or eight cells (figs. 2, 3). It was inferred that some of these tips must have passed the filter.

Additional samples were then filtered in our laboratory, and 100 cc. samples of this filtrate centrifuged for three minutes to separate out any

tips that might have passed through the filter. Samples drawn from the bottom of the centrifuge tube after this treatment showed, when examined with the compound microscope, that a few of these tips had been drawn through the filter. The tips in the filtrate were mostly single-celled but a few were as much as three cells long. They could not be detected in the filtrate with the unaided eye.

Experiments begun by us to study this growth more closely showed unexpectedly that the very tip points of a great number of these cells were glutinous and would instantly stick to any solid object touched. This was first noted when very young blades of grass were placed in cultures to determine if our plant would live on these. It was noted almost immediately that these small tip branches would stick to the grass, and even though these were moved around in water, the attached tips would sway about but would not become detached.

In a dish in which many of these tips are floating about, a string or blade of grass after being drawn through the water will, upon examination under the compound microscope, show a number of these tips sticking to it. This glutinous property possessed by some of these cell-tips was further demonstrated when water containing many of them was placed under the high magnification of the binocular microscope. A needle touched to the tip of these branches then drawn through the water revealed that the tip-branches would be drawn along without becoming detached. Considerable shaking about in the water was often necessary to dislodge them. Not all of these tip-branches possessed this glutinous quality but most of the several-celled ones did, as did also a number of the single-celled ones. These short single-celled branch tips were the type that most frequently passed through the filter.

When left in a petri dish undisturbed for a few hours, a number of these tips can be seen attached to the bottom of the dish by the point end and standing upside down. Agitating the water only causes them to sway around the point of attachment but does not loosen them. After a few days, when a few granules of sugar were added, these plants were observed to have formed a holdfast system from this basal pointed cell, the upper part had grown considerably and had become branched, and these small plants were now rapidly assuming the appearance of more mature ones. By growing in this way, the polarization of growth is reversed.

Under normal conditions in nature, it seems obvious that these branch-tips, after becoming free in the water, are carried by the current

until the pointed tip cell comes in contact with some object in the stream. They then stick and proceed with their development. On a firm surface the basal cell soon forms a holdfast apparatus by flattening out and branching (figs. 9, 10, 11, 13, and 14). Later, other branches are given off by a few cells immediately above, which likewise grow downward, become attached to the surface and thus form a very substantial holdfast system (fig. 7 and 8). In mature plants this holdfast system reminds one of the prop roots developed by Indian corn. On soft tissue such as young tender grass shoots, these young plants may send their first simple holdfasts into the tissue of the substratum (fig. 12), but that this growth is not for nourishment is shown by the fact that the plant will not grow unless sugar is added. When sugar is present, growth is as active on rocks and glass as on organic material.

CULTURE EXPERIMENTS

Since the chemical analysis of the branch water in which the plants were growing showed the presence of a considerable amount of glucose sugar, our first effort to culture this plant was in a sugar solution. The blades of grass to which the material was attached were divided into three approximately equal parts and placed in fingerbowls in distilled water. One of these was left on the desk at room temperature, one placed in the icebox but no sugar added, and the third placed in the icebox and a few grains of sugar (dextrose) added each day. The culture to which sugar was added began to grow immediately and after three weeks had reached its maximum growth. Before any sugar was added the growth of the plant extended out about one centimeter from the blade of grass to which it was attached and at the end of this period when measured again, had increased to about 2½ cm. Soon after this the culture had to be disposed of because of excessive bacterial contamination.

After about a week, the culture which had been placed on the desk had become completely destroyed by bacterial activity. In the icebox the bacterial action was retarded by a lower temperature so that, although the culture to which no sugar had been added showed very little if any growth, it was not completely destroyed after the three-week period.

Two weeks later more fresh material was collected and additional cultures made using several concentrations of dextrose in the water. The amount of sugar used was 3.5, 7, 10, 14, and 20 mg. per liter of distilled water. In all of these concentrations except the 3.5 mg. per

liter solution the growth was discernible after a week's time, but bacteria could not be eliminated from cultures made in this way so they had to be discarded. No further culture attempts were made until after October 15, 1938, when repeated efforts finally resulted in obtaining growth on agar that was entirely free of bacteria.

After we had obtained good growth of bacteria-free cultures on agar, we undertook again to try culturing this plant in a solution. Because growth was relatively good on maltose-peptone #5 agar, maltose-peptone solutions were made up without the agar. Solutions of 0.2 and 0.3 per cent dextrose were also prepared and these were placed in two liter Erlenmeyer flasks and autoclaved. To each flask was added a small block of an agar culture containing fresh growth on December 1, 1938.

After 24 hours, these showed that the plant had grown out quite noticeably from the block of agar that had been used for the inoculation, and when held up to the light, showed numerous tip cells floating about in the solution. Very few of these tips were seen at this time in the 0.2 and 0.3 per cent dextrose solutions as compared to the number in the maltose-peptone #5 medium.

After three days, the maltose-peptone solution began to appear slightly milky, and when held up to the light, the solution was found to be full of these minute tips floating about. A few had already settled to the bottom of the flask and were beginning to branch, as were many of those floating about in the solution. The flasks containing the dextrose solutions never became so full of these floating tips nor was such an abundant growth produced later as took place in the maltose-peptone solution. These tip branches continued to grow and, as they increased in size, settled to the bottom of the flasks.

This growth continued unobserved for several weeks during the Christmas recess, but after a month's time, the bottoms of the flasks were found covered to a depth of a little over an inch with the white fluffy growth of this plant (fig. 21). Most of the dividing branch-tips had also settled to the bottom so that the upper part of the solution no longer had a milky appearance. After this very little change was noted in the amount of growth, and by March 1, 1939, the only difference seen was a collection on the bottom of the flasks of a very slightly brownish mass somewhat more dense than the upper more fluffy portion. When this was examined it was found to be only a dense aggregation of short fragmenting branch-tip cells.

The appearance of the plants grown in the maltose-peptone #5

solution is quite similar to that of plants found in nature. The branching is very much the same and the general structure appears to be identical. However, some slight variation has been noted in the shape of the cells. The cells on the main axis of these plants show considerable enlargment at the nodes as had been noted before in cultures that had become badly contaminated with bacteria (figs. 5, 25). The cells of the side branches also frequently show considerable enlargement at the nodes, particularly on the end of the cells at which branching takes place. This type of enlargement gives these cells somewhat of a drumstick appearance (fig. 15). The cells of the side branches continually produce one to several-celled branches which are liberated into the solution.

The behavior of these reproductive tip-branches is different in solution from those of material brought in fresh from the stream. None of these tips seem to possess sticky points and could not be induced to stick to a needle drawn through the water, as could those brought in from the field. They also do not become attached to the glass but either continue to grow in the solution or fragment to form a number of shorter cells which grow into multicellular branched plants or just increase in size and fragment again. This fragmentation takes place after the cell has divided by the rounding up of adjoining ends of the two new cells (fig. 18). This rounding of the ends of the cells continues as the cells enlarge so that the two new cells either separate entirely or remain attached to each other with the ends continuing to elongate. Because of this tendency to stick together, several cells are frequently found which remain attached to each other but which are growing and dividing as if growing separately. Often before branching takes place in one of these branch tips, the cell at one end becomes very long and thread-like (fig. 17), suggesting perhaps an attempt at holdfast formation. Sometimes the tip cells on much branched plants in solution will become elongated and thread-like in a similar fashion before being set free by the parent plant (fig. 16).

GROWTH ON AGAR

In our early attempts to obtain growth of this plant on agar, the material was so completely polluted with bacteria that all efforts to obtain it in pure culture failed. In a later attempt, some plants began to grow along with a yellow bacillus-type bacterium which seemed to interfere in no way with the growth of the plant. Eventually pure cultures free of bacteria were obtained by cutting off tips of branches which had grown

away from bacteria under the surface of the agar. When we first attempted to grow cultures on a solidified medium, 3% agar was used for this purpose. When this failed to give good results, the possibility of the medium being too solid was suggested. When the amount of agar was reduced to 1.2%, growth was more satisfactory. Potato-dextrose, Blakeslee's #230, corn meal, 0.2% dextrose, and our maltose-peptone #5 agars were all prepared with this concentration of the agar. The potato-dextrose and Blakeslee's #230 seemed too strong and no growth took place on either of these. Fair growth resulted on the 0.2% dextrose but the best results were obtained on the maltose-peptone #5 agar.

As soon as reproductive tips are placed on agar, the cells divide and begin to grow. After 24 hours, many have become several-celled and have even begun to branch in some cases. The tip cell elongates, becomes narrow and much bent without forming any crosswalls, and grows down into the agar. This tip cell is the one which would have given rise to the holdfast system had this branch become attached to some object in water. Each small plant thus started continues to grow on the agar, the cells enlarge and branches arise from the anterior end of each cell near the joint with the adjoining cell as do the plants found in nature in the running water. These branches very seldom grow out and become more branched as do the hyphae of cultures of fungi, but rather grow more parallel to the main axis, become separated from the parent cell, and are pushed aside by another branch from the same cell. Many cells are, therefore, produced along the main axis which lie parallel with it. Each cell or group of cells that formed a branch in this way continues to grow and produce branches as did the first axis. In this way whole bands of branching strands are formed in which the larger axis is in the middle and the smaller branching side axes are found around this (fig. 23). The large cells of the central axis are very conspicuous in a band of growing threads.

Growth on agar may be either on the surface or under the surface (fig. 22). Growth on the surface produces a raised, white, glistening colony with the greater amount of growth at the point of inoculation with raised bands extending outward. Under the agar the bands are not quite so compact. A surface band always is accompanied by some growth down into the agar. Whether the growth is on the surface or down in the agar, the main axis does not extend straight but seems to meander somewhat in a zig-zag fashion. This is perhaps due to the plant's growth against the resistance of the agar and the elongation of

each cell in place after it was first cut off. This winding growth is very evident in surface growth and is shown clearly in fig. 23. If one of these surface strands is removed and placed in a drop of water, the plants straighten out and appear almost exactly as plants removed from their attachments in the field. They have no holdfasts, of course, but otherwise there is no detectable difference.

Frequently when branches from a colony grow downward through the agar, they attempt to form holdfasts against the bottom of the petri dish (fig. 26). These holdfast-like branches, however, are seldom divided into cells and do not approach the more complex holdfast apparatus of plants grown attached to objects in water. Holdfast-like structures have not been observed formed in the agar except where a growing point has touched the bottom of the petri dish.

The growth of this plant on agar is very slow. Pure culture transfers made by cutting out small blocks from an original agar culture or by transferring a strand of the surface growth, may produce a colony in a month which rarely has a radius of more than an inch from the point of inoculation. The white glistening accumulated growth in the center of these colonies is very suggestive of the appearance of bacteria or yeast colonies.

COMPOSITION OF THE CELL WALL

The cell wall was first tested for cellulose by the usual chloro-iodide of zinc treatment. A few plants were placed on a slide in a drop of water and with them a few hyphae of *Achlya flagellata* and cotton fibers, the walls of which show perfectly the cellulose reaction with this solution. When the water was drawn off, and the reagent added, the walls of our plant became only faintly yellowish while the hyphae of the *Achlya* and the cotton fibers turned a beautiful violet-blue color. As a further test, the procedure outlined by Chamberlain (Methods in Plant Histology, 5th revised ed., p. 85) for demonstrating the presence of cellulose was followed. Here again the walls of our plant yielded only a yellowish color while the walls of the *Achlya* and the cotton fibers became a deep blue. These two tests indicated to us that cellulose was either not present in the walls of this plant or that these tests for some reason were prevented from indicating it.

We then tried Zander's test for chitin as given in Lee (Microtomist's Vade-Mecum, 10th ed., p. 600) but this failed to give a positive reaction. When treated with gentian violet, the walls became stained with this dye.

Samples of our plant brought in from the stream and carefully cleaned, and samples taken from the surface of agar cultures were dried and very generously analysed for us by Dr. J. C. Andrews of the Department of Biological Chemistry. We are indebted to Dr. Andrews for the following results: Hydrolysis gives glucose, and the glucosamine test was negative. When tested for nitrogen 2.80% was obtained. This would correspond to a maximum of about 36% chitin if *all* N were in that form. This would, likewise, correspond to a maximum of about 17.5% protein.

This material gave a positive biuret test and positive tests for tyrosine and tryptophane. A test for cystine showed only a very slight amount of this substance.

The above results indicate that the walls probably do not contain chitin but rather a carbohydrate related to cellulose, the composition of which is at present unknown.

TAXONOMY

In regard to its relationships to other algae, an extended discussion seems unnecessary. Except in this case, there is no saprophytic plant known that can be convincingly compared to any Green Alga group. It is true that Printz in Engler and Prantl (Pflanzenfamilien, 2nd ed. **3**: 252. 1927) suggests the Monoblepharidaceae as colorless relatives of the Oedogoniaceae, but if there is any such relationship, it is more than obscure. A comparison of our plant with parasitic and endophytic algae suggests no parallels. It may be of significance that *Stigeoclonium tenue*, a green plant, has been found attached to living fish in a fishpond at Melbourne, Australia, and in a small pool at Tanabe, Japan (Tilden, Algae and their Life Relations, p. 458).

The peculiar nature of this plant makes it extremely difficult to fit into any scheme of classification. From its vegetative structure it would seem best to consider it a highly peculiar derivative of the algae, with its nearest relatives in the higher forms of the Chaetophoraceae. The differences would seem sufficient to place it in a family of its own, which might best be placed near the Chaetophoraceae as an aberrant member of the order Ulotrichales.

SAPROCHAETACEAE n. fam.

Colorless saprophytic plants with branching and general form of the Chaetophoraceae; attachment to substratum by means of a complicated rhizoidal holdfast system; reproduction, as far as known, solely by a vegetative detachment of the branch tip cells.

One genus, *Saprochaete*.

SAPROCHAETE n. gen.

With the characters of the family. One species.

Saprochaete saccharophila n. sp.

Plants occurring attached to objects in running water, appearing milk white in mass, reaching a total length usually of about 2 cm., under some circumstances up to 5 cm. Plant body complicately branched from an attached base, branches arising from the upper ends of the cells, ultimate branchlets tapering to a sharp point; axis cells up to 175μ long by 33μ in diameter, cylindrical, sometimes having enlargements at the cell junctions, nuclei in largest cells conspicuous, up to 8.6μ in diameter and having a prominent nucleolus; chlorophyll and plastids entirely lacking; cell wall not responding to tests for cellulose or chitin; propagation entirely by vegetative means, the distal cell or cells of the branchlets becoming free, then attaching themselves by their glutinous tips and growing backward, reversing polarity.

Found only in running water in which a considerable amount of reducing sugar is present. Collected many times during cooler months from the spring of 1937 to 1939 at two stations in Chatham County, N. C., growing attached to objects in spring runs receiving seepage from burning sawdust piles.

SUMMARY

A remarkable plant having the form of a higher green alga and the nutrition of a saprophytic fungus is reported from two stations in northern Chatham County, N. C., growing in small spring runs receiving seepage from burning sawdust piles. It has a body closely resembling that of *Stigeoclonium*, but is entirely without chlorophyll or any trace of an organ resembling a chloroplast. The cytoplasm is confined to an extremely thin layer under the wall in the larger cells, and each of these cells has a single large conspicuous nucleus. The tip cells of the branches are extremely tenuous and end in a fine point which has been found in most cases to be viscid. These tip cells fall off easily and become attached by their points to objects in the water. Reversing their polarity, they soon develop into much branched plants reaching a length of 1 cm. or more and having the appearance to the naked eye of a vigorous water mold. This method of vegetative multiplication is the only type of propagation known for this plant so far. A complicated group of rhizoidal holdfasts is sent off from the basal cell and the cells above it. The cell walls do not give the usual positive reactions for either cellulose or chitin with standard tests, but a chemical analysis indicates a closer affinity to cellulose.

Pure culture isolations have been grown on agar containing small amounts of sugar, and in weak sugar solutions. The appearance of the growth in culture and any deviations from the typical structure of plants found in nature are discussed. For nutrition, this plant seems to be dependent on the presence of reducing sugars, as is indicated by its growth in nature in water in which reducing sugars are present and from cultural studies in sugar solutions in the laboratory.

As far as the authors are aware, no saprophytic plant with the form of a multicellular green alga has previously been reported. This plant is being placed in a family of its own, the Saprochaetaceae, near the Chaetophoraceae, and given the name of *Saprochaete saccharophila.*

DEPARTMENT OF BOTANY,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

EXPLANATION OF PLATES

PLATE 22

Fig. 1. Habit sketch of a single plant. × about 18.

Fig. 2. End of branch showing several of the type of branch-tips used for vegetative multiplication. × 122.

Fig. 3. Single branch-tip after separation from parent plant. × 368.

Fig. 4. Outline of a few cells of a main axis showing position of branches. × 368.

Fig. 5. Outline of cell of a main axis from plant taken from a contaminated culture kept in the laboratory to show enlargement at ends of cells (drawing by Miss Alma Holland). × 335.

Fig. 6. Surface view of cell from plant killed in the fumes of osmic acid and stained with gentian violet showing the large nucleus and the appearance of the cytoplasm. × 368.

Figs. 7 & 8. Basal portion of older plants scraped from grass showing holdfast system that attaches plant. The ends of the holdfast branches have been broken off. Fig. 8, a, shows a young plant starting to grow upward from the holdfast branch of the parent plant. × 368.

Figs. 9–11. Early attachment stages of young plants on hard surfaces showing the flattening and branching of the tip cell. × 368.

Fig. 12. Stage showing the formation of a somewhat rhizoidal structure in the attachment of a branch-tip to a soft tender grass shoot, g. × 368.

Figs. 13 & 14. Young growing plants on tender grass blades. × 368.

Fig. 15. Branch from main axis of plant grown in maltose-peptone #5 solution showing peculiar enlargements at ends of cells. × 368.

Fig. 16. Tip of branch showing elongate thread-like growth of end cells which is very frequent either when growing in solution or on agar. × 368.

Fig. 17. Branch tip from maltose-peptone #5 solution showing threadlike elongation of one end cell and the breaking away of a small cell at the other end. × 368.

Fig. 18. Separating small cells from dividing branch-tips taken from maltose-peptone #5 solution. $\times$ 368.

PLATE 23

Fig. 19. Photograph of rocky stream bed showing thick mat of fluffy growth covering the rocks.

Fig. 20. Blades of grass with fluffy attached growth. $\times \frac{5}{6}$.

Fig. 21. One month's growth in maltose-peptone #5 solution with considerable accumulation of fluffy growth on the bottom of the flask. $\times$ about $\frac{1}{3}$.

Fig. 22. Growth on agar one month after inoculation. Note accumulated surface growth in center of colony and zig-zag radiating strands. $\times 1\frac{2}{3}$.

Fig. 23. Termination of surface strand. Note bending and meandering type of growth and the large central axis in center with side branches running about parallel to this. $\times$ 92.

Fig. 24. Basal part of plant removed from blade of grass showing stubs of holdfast branches. $\times$ 67.

Fig. 25. Cell of a main axis from material kept in the laboratory for a few days. Note enlargements at ends of cells and the position of the origin of the side branches. Arrow points to nucleus with its large nucleolus. Unstained. $\times$ 475.

Fig. 26. End of branch that has grown down through the agar and is attempting to form a holdfast against the bottom of the petri dish. $\times$ 120.

PLATE 22

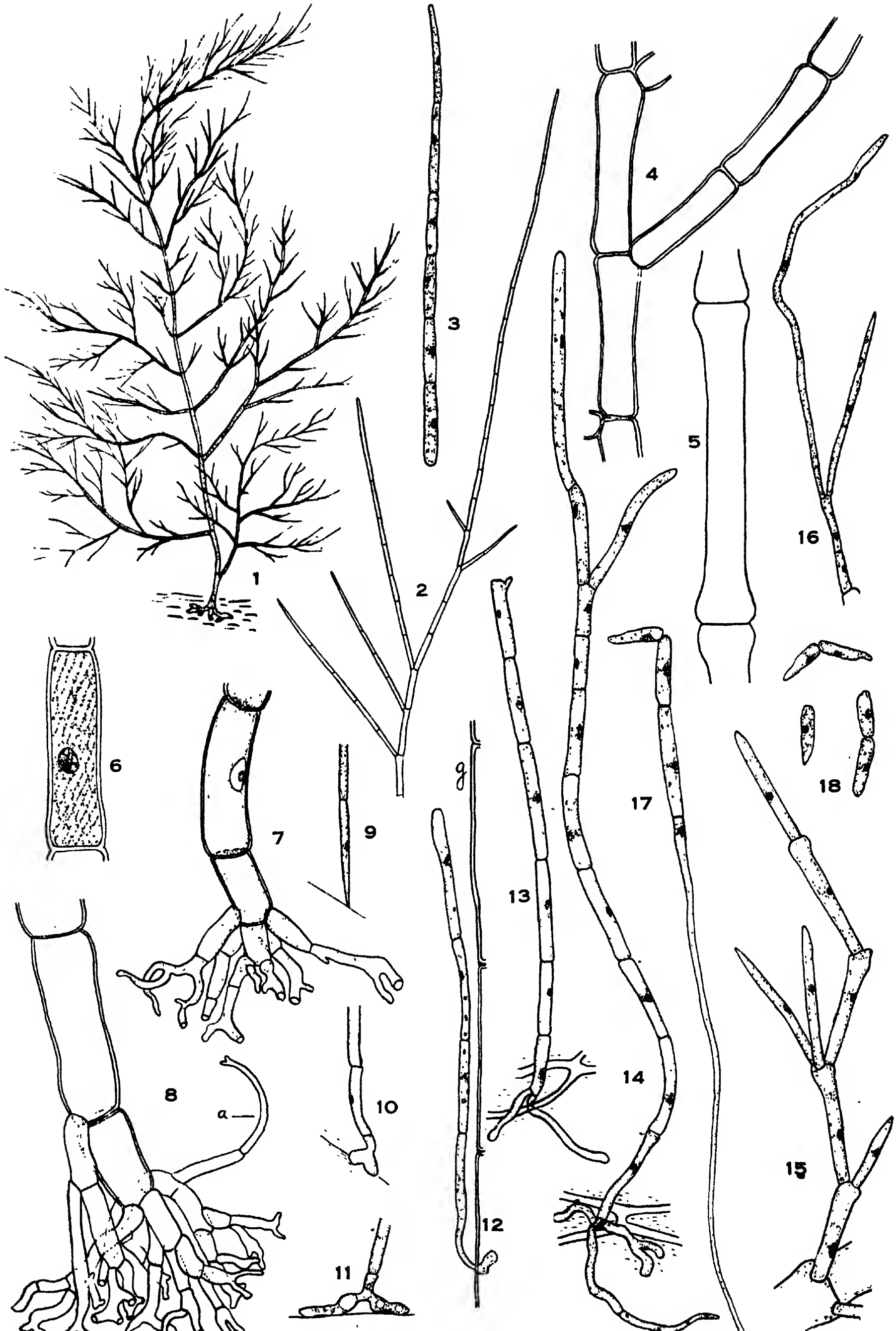
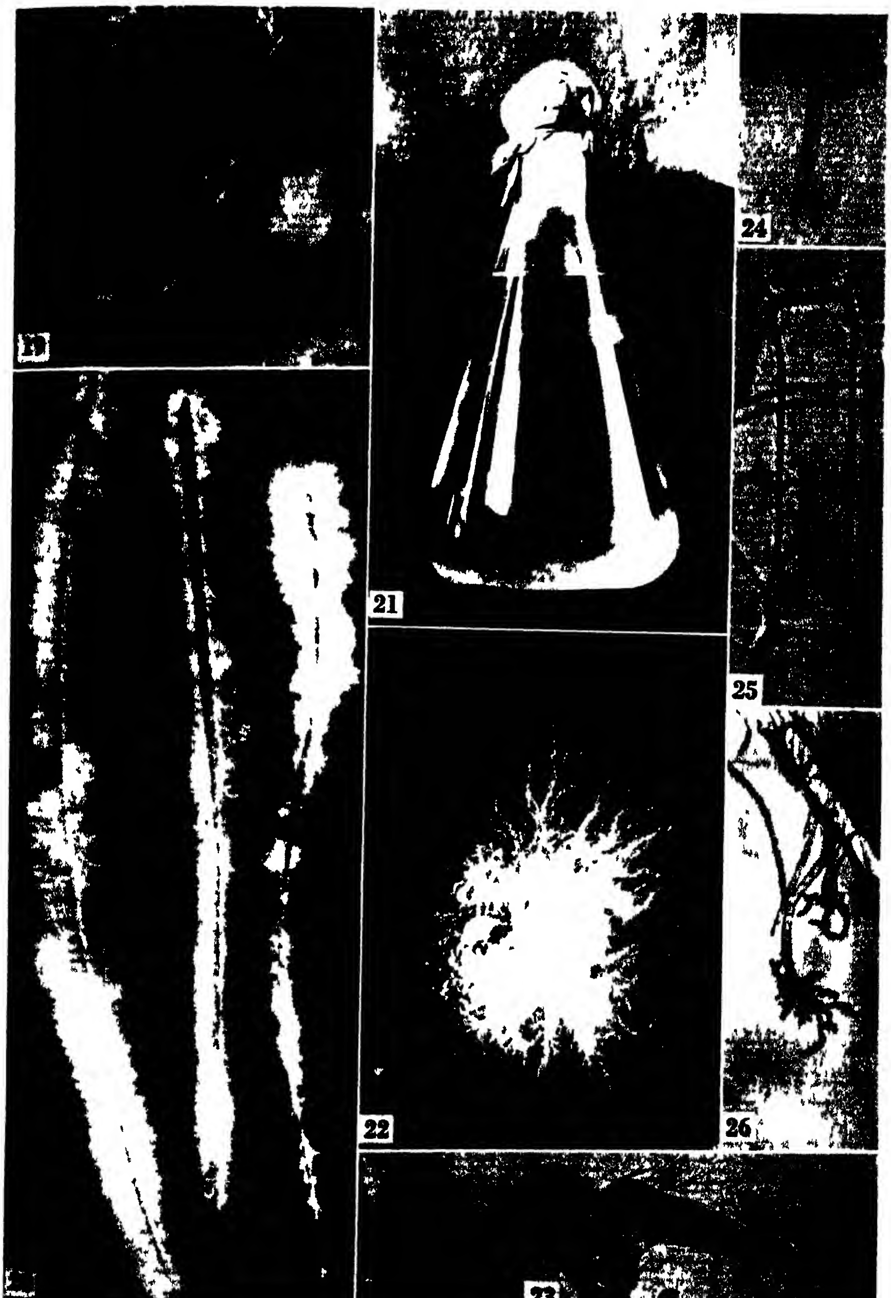


PLATE 23



STUDIES IN THE GENUS OLPIDIOPSIS. I

Resting Spore Germination in a New Species*

By Leland Shanor

Plate 24 and One Text Figure

Observations on resting spore germination in what is now considered to be an authentic species of *Olpidiopsis* apparently are not described in the literature. Fischer (1880, 1882) has described the germination of what he considered at that time to be the resting spores of *Olpidiopsis Saprolegniae*. The resting spores in this case, however, were not accompanied by the companion cell that is characteristic of this genus, so Fischer later (1892) decided that the plant that he had been working with before was a member of an entirely new and distinct genus for which he proposed the name *Pseudolpidium*. The chief difference in the two genera is the lack of a companion cell on the resting spores of *Pseudolpidium*.

Zopf (1884) has described rather briefly resting spore germination for his *Olpidiopsis Schenkiana*. Zopf's plant, however, possesses uniciliate zoospores rather than the biciliate type characteristic for *Olpidiopsis* and has been transferred to the genus *Pseudolpidiopsis* by von Minden (1915). Scherffel (1925) considers Zopf's observations of the number of cilia possessed by the zoospores of *O. Schenkiana* incorrect and reports this species to have biciliate zoospores. Tokunaga (1933,a) has since observed a form occurring on *Spirogyra* in Japan, considered to be *Pseudolpidiopsis Schenkiana* (Zopf) v. Minden, whose zoospores, he says, possess "a long cilium." There is a possibility, therefore, that

* This paper was presented before the Mycological Society of America, meeting at Richmond, Va., December, 1938, under the title "An Interesting Type of Resting Spore Germination in *Olpidiopsis*." The author wishes to express his sincere appreciation to Dr. W. C. Coker for his helpful suggestions during this study, and to Dr. J. N. Couch for carrying on the stock cultures during his absence from the University, June to September, 1938. This and the following paper are taken from material submitted to the Graduate Faculty of the University of North Carolina as a dissertation for the Ph.D. degree in the Department of Botany.

two very similar parasites occur on *Spirogyra* and that the observations on the number of cilia by all of these investigators is correct. Fitzpatrick (1930) follows von Minden in recognizing the correctness of separating the forms possessing uniciliate zoospores from those possessing biciliate zoospores.

Gäumann and Dodge (1928) cite Barrett (1912) as saying that the resting spores of *Olpidiopsis* germinate by zoospores which are discharged through an emission collar. Barrett (1912), however, states that as yet he had not observed cases of "oospore" germination in any of the species which he had studied, and gives, as the only cases in which it had been described up until that time, those by Fischer (1882) for *Olpidiopsis* (*Pseudolpidium*) and by Zopf (1884) for *Olpidiopsis Schenkiana* (now *Pseudolpidiopsis Schenkiana* (Zopf) v. Minden). In another species of *Olpidiopsis* occurring on an alga, *Olpidiopsis oedogoniorum* (de Wildeman) Scherffel, Scherffel (1925) states that resting spore germination is unknown.

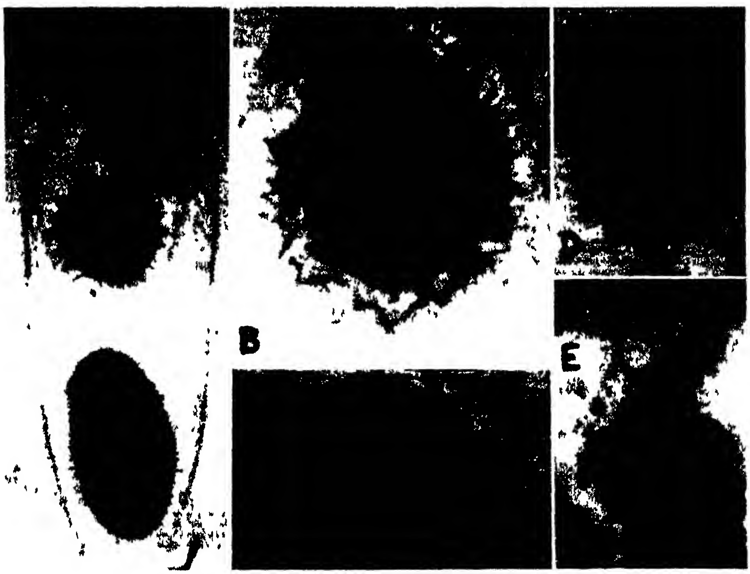
Gwynne-Vaughan and Barnes (1927), in summarizing the information known about *Olpidiopsis*, state that "Nothing is known about the fate of this resting structure, to which the empty antheridium remains firmly attached," but in a later edition (1937) suggest that "the oospore probably discharges biflagellate zoospores when it germinates." Smith (1938) states concerning this genus that "Germination of the zygote has not been observed." That the assumption of Gwynne-Vaughan and Barnes is correct is shown by the observations recorded in this paper.

MATERIAL STUDIED, INCLUDING THE DESCRIPTION OF A NEW SPECIES

The material from which these observations were made was collected from a roadside pool near Burgaw, North Carolina, March 18, 1938, parasitizing *Achlya flagellata* Coker. At this time the plant was carefully studied and resting spore germination looked for, but up until June no cases were observed in any of the cultures. At the close of the school term in June, new cultures were made and those were left with Dr. J. N. Couch to be kept going over the summer months. Upon my return in September, numerous empty resting spores with ruptured germ tubes and a number of others in the process of germination were found in the old cultures. The process was then carefully followed a number of times and the observations are given below.

This species of *Olpidiopsis* does not agree with any known species.

The companion cell to the resting spore is, in most cases, distinctly spiny, due to the fact that the spiny encrusting exospore wall that covers the resting spore is quite thick and also encloses the companion cell (Text figs. B. & C.). Some of the smallest resting spores may have smooth-walled or practically smooth-walled companion cells (fig. 12).



A. Enlargement of host hypha containing two typical spiny zoosporangia and one resting spore. × 160. B. Large resting spore before fertilization showing protoplasm in the antheridium Spiny wall of the companion cell barely showing as it is partly masked by the disintegrating host protoplasm × 340. C Smaller resting spore in the dormant condition. Note the prominent spiny layer continued over the companion cell and outer dense and inner less dense regions of the resting spore protoplasm × 340. D Early stage in resting spore germination. Note hyaline tip of germination tube and clearer vacuolar region × 250 E. Stage in germination just previous to zoospore formation. Note absence of vacuole and the presence of refractive globules in the now more or less homogeneous protoplasm; also shape of germination tube caused by conformity to the shape of the companion cell. × 250.

The manner in which the spiny layer is laid down is probably responsible for this variation, as there is often some variation in the sharpness of the spines of the exospore wall on the resting spores also.

Cornu (1872) has described the resting spores of *Olpidiopsis index* as having a spiny companion cell, but the spines on his plant are very

slender and are quite different from those of this fungus. The spines on the resting spores of this new species are rather broad at the base and are more like those possessed by *Olpidiopsis minor* Fischer. The companion cells of *O. minor*, however, are smooth rather than spiny. The resting spores of *Olpidiopsis minor* are also usually much smaller. Butler (1907), however, says that in the material studied by him they were larger, being about the same size as the resting spores of *O. Saprolegniae.* I have been fortunate enough to have cultures of *O. minor* for comparison and our plant seems to be too distinct to be even a variety of that species. The elliptical resting spores so frequently formed in cultures of *O. minor* have never been found in any cultures of this new fungus. In this plant resting spores are always spherical. Tokunaga (1933) has described a species from Japan, *Olpidiopsis spinosa,* in which both the resting spore and the companion cell are beset with long slender spines. Tokunaga's species is quite distinct in this respect. The average size of the resting spores of our new *Olpidiopsis* closely approaches that of *O. Saprolegniae* Cornu (not *O. Saprolegniae* (Cornu) Fischer), but the character of the spines on the two species is quite different and the companion cell of *O. Saprolegniae* is smooth-walled. The hyphal enlargement typically produced by *O. Saprolegniae* is also mostly terminal and frequently more spherical, whereas that of our plant is often intercalary and, when terminal, the enlargement is much more elongate. *Olpidiopsis index* Cornu and *O. spinosa* Tokunaga are the only other species known in which the companion cell is not typically smooth-walled.

Another very interesting variation from the usual situation reported for the genus is the presence of spines on the zoosporangia. The zoosporangia range from perfectly smooth-walled forms to ones densely covered with sharp spines (Text fig. A), but by far the greater majority have spines of some type. All imaginable intermediate gradations of spininess can often be found in one heavily infected hypha. The germination of both the smooth and spiny zoosporangia is identical and neither shows any more of a resting tendency than does the other. The spines on the zoosporangia are apparently of the same structure as those on the resting spores. The spines in neither case give a cellulose reaction with chloro-iodide of zinc, whereas the walls of both smooth and spiny zoosporangia are cellulose.

At first these spiny zoosporangia were suspected by the author to be the "Dauerspores" of Fischer's genus *Pseudolpidium,* but single spore isolation cultural studies have shown that they belong to this

Olpidiopsis. They certainly fit the descriptions of the resting spores of *Pseudolpidium*, particularly of *P. Saprolegniae* but sometimes of *P. fusiforme*, and appear remarkably similar to the figures found in the literature for this genus. Sparrow (1932) has reported that some zoosporangia of *O. minor* Fischer possess sharp scattered spines, but does not mention a resemblance to the resting spores of *Pseudolpidium*.

Because of the fact that there is so much variation in the spininess, not only of the sporangia but also of the resting spores, it might appear to one not studying this plant in detail that several species of the genus might be contained in a vigorous hyphal infection. This great variability makes the name *Olpidiopsis varians* seem appropriate. The description of this new fungus follows:

Olpidiopsis varians n. sp.

Zoosporangia single to many, formed either in terminal or intercalary swellings of the host hypha, elliptical, oval, or spherical, extremely variable in size from 60 by 40μ up to 350 by 140μ, frequently about 200 by 80μ, walls giving cellulose reaction with chloro-iodide of zinc, smooth to very spiny, spines slender and somewhat conical, up to 7μ long, not giving the cellulose reaction; exit tubes commonly one to three (as many as five have been observed). Zoospores oval to elongated, 3.8–4.6μ long by 2.3–3.0μ in diameter, usually about 4.2 by 2.8μ, biciliate, cilia of about equal length, measuring from about 4.2 to 4.6μ. Oogonia yellowish-brown and very variable in size, spherical, with usually one but sometimes two antheridia attached, 26 to 83μ in diameter (not including spines), averaging between 52 and 61μ; exospore wall colorless to yellowish, about 1.2μ thick, bearing usually coarse abruptly tapering spines which measure up to 8.6μ long and have a reticulum connecting them, not giving the cellulose reaction; endospore wall yellowish-brown, about 1.7μ thick, smooth, and giving the cellulose reaction. Antheridia (companion cells) usually spherical, 17 to 30μ in diameter, commonly about 26μ, wall occasionally smooth but typically covered by scattered spines similar to those on the oogonia but much shorter, 1.7μ at the longest, outer part of wall bearing spines usually colorless, inner wall having a slightly yellowish cast. Contents of antheridia pass into oogonia and antheridia on mature oogonia are empty. Germination takes place in this species by means of a germination tube which usually penetrates the companion cell. Biciliate zoospores are produced when the resting spore germinates.

Zoosporangiis solitariis vel numerosis, in cellulis terminalibus vel intercalaribus matricis formatis; ellipsoideis, ovalibus, vel sphaericis, valde variis, 60–350 x 40–140μ, saepissime 200 x 80μ; membrana, quae reactionem cellulosae dat, levi vel valde echinulata, spinis anguste conicis, longis ad 7μ, cellulosa carentibus; zoosporis ovalibus vel elon-

gatis, 3.8–4.6 x 2.3–3μ, saepissime 4.2 x 2.8μ, biciliatis, ciliis equalibus in longitudine, 4.2–4.6μ. Oogoniis luteo-brunneis, valde variis in magnitudine, sphaericis; antheridio uno vel duobus, 26–83μ in diam. (praeter spinas), saepissime 52–61; episporio hyalino vel luteo, 1.2μ crasso, spinis crassis acutis, longis ad 8.6μ, cellulosa carentibus; endosporio luteo-brunneo, 1.7μ crasso, levi, cellulosam habente. Antheridiis sphaericis, 17–30μ in diam., saepissime 26μ, levibus vel plerumque echinulatis, spinis longis ad 1.7μ.

Collected on *Achlya flagellata* Coker, from a roadside pool near Burgaw, N. C., March 18, 1938.

A detailed description of the germination of the zoosporangia of this species is unnecessary since the stages in maturation and germination are essentially the same as those described for other species by Barrett (1912), and for the zoosporangia of *Pseudolpidium Aphanomycis* by Butler (1907).

OBSERVATIONS ON MATURATION AND GERMINATION OF THE RESTING SPORES

After fertilization has taken place and the resting spore has formed an endospore wall about itself, the protoplast soon presents the mature structure. In the mature condition the resting spore has a very characteristic appearance. There is an outer region of very fine, densely granular protoplasm that is separated rather sharply from a central region of coarser granular protoplasm. Often small oil globules may be located in either region. In this condition the resting spore may lie dormant for an indefinite period.

The first noticeable change to occur indicating that the resting spore is going to germinate is the disappearance of the sharp dividing limitation between the outer dense, finely granular region and the inner more coarsely granular one. As this takes place, the denseness of the protoplasm in the outer region diminishes and soon all of the protoplasm appears similar except for the presence here and there of small oil globules. A large vacuole has now appeared and lies somewhat in an eccentric position. Unless conditions are changed, such as the addition of fresh water to a culture, resting spores may remain in this condition for a rather long period also.

Just before the germ tube is initiated, a slightly more hyaline region appears just under the companion cell (fig. 2). Before long the wall between the resting spore and the companion cell is dissolved away and the growth of the germination tube into the companion cell takes place

(fig. 3, & Text fig. D). This growth continues until the germination tube reaches the opposite wall of the companion cell. The germination tube commonly widens after reaching the opposite wall so that usually the width of the germination tube is equal to the width of the companion cell (fig. 7 & 8). This is not always the case, however, and sometimes there is a distinct space between the germination tube and the wall of the companion cell (fig. 4, etc.). The wall of the germination tube gives the typical reaction for cellulose with chloro-iodide of zinc. Usually a short time now elapses before the germination tube can dissolve its way through the opposite wall of the companion cell. In the meantime, the vacuole usually changes its shape continually, shifting its position occasionally, and often now more small oil globules can be seen.

After dissolving its way through the companion cell wall (fig. 4), the germination tube grows to a short distance beyond before ceasing to elongate and often at this point germination may again be arrested. This is particularly true when the material under observation is under a cover glass. When germination proceeds again, the protoplasm becomes more coarsely granular, small refractive globules appear scattered throughout, and the vacuole disappears entirely (fig. 7 & 8, and Text fig. E). In a short time, the initials of the zoospores can be seen (fig. 5) and, in a few seconds after their formation, the zoospores set up a motion within the resting spore. This shuffling, jerky movement continues until finally the end of the germ tube is ruptured after having become softened. When the rupturing takes place, the spores nearest the end of the germination tube are discharged so forcibly that they do not come to rest until some distance from the tip of the tube. Instead of swimming away immediately after the force of the discharge carries them no farther, they remain stationary for a second or two, as if bewildered, and then swim away rapidly. The other zoospores in the resting spore keep shuffling about and the ones nearest the opening escape as rapidly as the opening in the end of the germ tube will allow. The opening in the end of the germ tube is not much larger than will permit the escape of a single zoospore at a time. Usually within a few minutes all of the zoospores make their escape (fig. 6). These zoospores produced by the germination of the resting spores appear identical to those produced by the germination of the zoosporangia. They possess two cilia which are attached laterally nearest the anterior end of the spore and swim with one cilium directed forward and having the other one trailing behind (fig. 6 & 6a).

DISCUSSION

After learning the secret of the necessary elapse of time after fertilization, I have been able to get germination of the resting spores in my cultures almost at will. All that seems necessary to do is to place in fresh water on a slide the resting spores from a properly aged culture. Those resting spores that have already proceeded as far as the approximate stage shown by Fig. 2, will continue germination almost immediately. These may be covered with a cover glass provided sufficient water is kept on the preparation to keep the cover glass floating. Almost immediately, then, the hyaline area will appear under the companion cell and the dissolution of the wall between commences. The whole process from the condition shown by Fig. 2, until zoospores are released may take place within half an hour. In other instances, some resting spores may produce a germination tube and then remain in this condition for a day or two before completing zoospore formation. This is particularly true when a preparation for examination is placed in the same water in which the culture was growing and additional water is added from the same source, or when the cover glass is not continuously kept floating during the observation. The process of germination seems to be hastened by the continued addition of fresh water to the material being examined.

Zopf (1884) found that he could obtain the germination of the resting spores of his *O. Schenkiana* by allowing the resting spores to dry on a slide for a few days and then adding fresh water to these. I have tried this method with several species of *Olpidiopsis* but have not as yet been successful in obtaining germination by this method alone. I have also tried freezing resting spores after fertilization but this has likewise failed to hasten the maturation process in preparation for germination. The resting spores in a culture about three months old that had been dry for some time germinated soon after water was added. Germination seems to take place only after the elapse of time necessary for the maturation to be completed normally. I have obtained resting spore germination in only this one species, and as yet, none of the resting spores of any of the other species at hand have shown any signs of germination.

The explanation for the fact that the germination tube grows through the companion cell in this species may be that there is less resistance to its growth at this point. At other places the resting spore is covered with a thick encrusting exospore that probably offers considerable

resistance to the progress of the germination tube's growth. Even in the case of the smallest resting spores this is the rule, and it seems always to be the case in the germination of the larger ones. It is sometimes rather difficult to determine with certainty whether the germination tube has grown through the companion cell in cases where the tube is formed before the observations were begun, but this can usually be determined after careful observation. When two companion cells accompany a resting spore, germination seems to take place through only one of them (fig. 10). Cases where more than one germination tube have been formed in the germination of the resting spores have not been observed.

SUMMARY

1. A new species of *Olpidiopsis* is described as *O. varians* n. sp. Most of the zoosporangia of this species possess spines of some description, but some of the zoosporangia are smooth-walled. A resemblance of the spiny zoosporangia of this species to the "Dauerspores" of Fischer's genus *Pseudolpidium* is noted. The companion cell of this plant is also spiny in the majority of cases, but the species is quite distinct from the other two known species which possess this characteristic.

2. Germination of the resting spores of an authentic species of *Olpidiopsis* is described, apparently for the first time. A long dormant period after fertilization has been found necessary before germination will take place. In this species there is usually formed a germination tube which penetrates the companion cell and biciliate zoospores are liberated. These show no apparent difference from the zoospores released by zoosporangium germination.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, NORTH CAROLINA.

LITERATURE CITED

1. BARRETT, J. T. 1912. Development and Sexuality of some Species of Olpidiopsis, (Cornu) Fischer. Ann. Bot. 26: 209–238.
2. BUTLER, E. J. 1907. An Account of the Genus Pythium and some Chytridiaceae. Memoir of Dept. of Agric. in India, Bot. Ser. I, No. 5, pp. 1–160.
3. CORNU, M. 1872. Monographie des Saprolegniées. Ann. des Sci. Natur., Bot., ser. 5, 15: 1–198.
4. FISCHER, A. 1880. Ueber die Stackelkugeln in Saprolegniaschlauchen. Bot. Zeit. 38: 689-96, 705–11, 721–26.

5. FISCHER, A. 1882. Untersuchungen über die Parasiten der Saprolegnieen. Jahrb. für wiss. Bot. 13: 286–371.
6. ——— 1892. Phycomycetes. Rabenhorst's Kryptogamenflora von Deutschland, I, Vol. 4.
7. FITZPATRICK, H. M. 1930. The Lower Fungi. Phycomycetes. McGraw-Hill Book Company, Inc., New York.
8. GÄUMANN, E. A., AND DODGE, C. W. 1928. Comparative Morphology of Fungi. McGraw-Hill Book Company, Inc., New York and London.
9. GWYNNE-VAUGHAN, H. C. I., AND BARNES, B. 1927. The Structure and Development of the Fungi. Cambridge Univ. Press, London.
10. ——— 1937 Ibid, Second Edition.
11. MINDEN, M. VON 1915. Chytridiineae. Kryptogamenflora der Mark Brandenburg 5: 209–422.
12. SCHERFFEL, A. 1925. Endophytische Phycomyceten-Parasiten der Bacillariaceen und einige neue Monadinen. Ein Beitrag zur Phylogenie der Oomyceten (Schröter). Archiv. für Protistenkunde 52: 1–141.
13. SMITH, G. M. 1938. Cryptogamic Botany. Vol. I, Algae and Fungi. McGraw-Hill Book Company, Inc., New York and London.
14. SPARROW, F. K. Jr. 1932. Observations on the Aquatic Fungi of Cold Spring Harbor. Mycologia 24: 268–303.
15. TOKUNAGA, Y. 1933. Studies on the aquatic chytrids of Japan. I. Woroninaceae. Trans. of the Sapporo Nat. Hist. Soc. 13: 20–28.
16. ——— 1933, a. Studies on the Aquatic Chytrids of Japan. II. Olpidiaceae. Trans. of the Sapporo Nat. Hist. Soc. 13: 78–84.
17. ZOPF, W. 1884. Zur Kenntniss der Phycomyceten. I. Zur Morphologie und Biologie der Ancylisteen and Chytridiaceen. Nova Acta K. Leop. Carol. deutsch. Akad. der Naturf. 47: 143–236.

EXPLANATION OF PLATE 24

Fig. 1. Dormant condition of the resting spore. Protoplasm in two rather sharply defined regions, the outer one finely granular and dense, the inner one more coarsely granular and less dense. × 368.

Fig. 2. Early stage in germination. Protoplasm is more homogeneous and much less dense than in the dormant condition. Note hyaline region just under companion cell. × 368.

Fig. 3. Germ tube growing into companion cell. The hyaline tip is still quite prominent. × 368.

Fig. 4. Germ tube has dissolved its way through companion cell and now the protoplasm is becoming more coarsely granular. × 368.

Fig. 5. Stage just previous to rupture of germination tube. Spores are almost completely formed and are in a very compact mass. Each spore contains one or more small refractive globules. × 368.

Fig. 6. Stage showing ruptured germ tube and escape of last zoospores. × 368. Fig. 6a, Zoospores killed in fumes of osmic acid and stained with gentian violet. × 1135.

Fig. 7. Stage intermediate between stages shown in Figs. 4 & 5. Protoplasm is more coarsely granular and numerous small refractive globules are present. Large vacuole is now absent. Basal part of germination tube completely fills companion cell. × 368.

Fig. 8. Smaller resting spore in same stage as shown in Fig. 7. × 368.

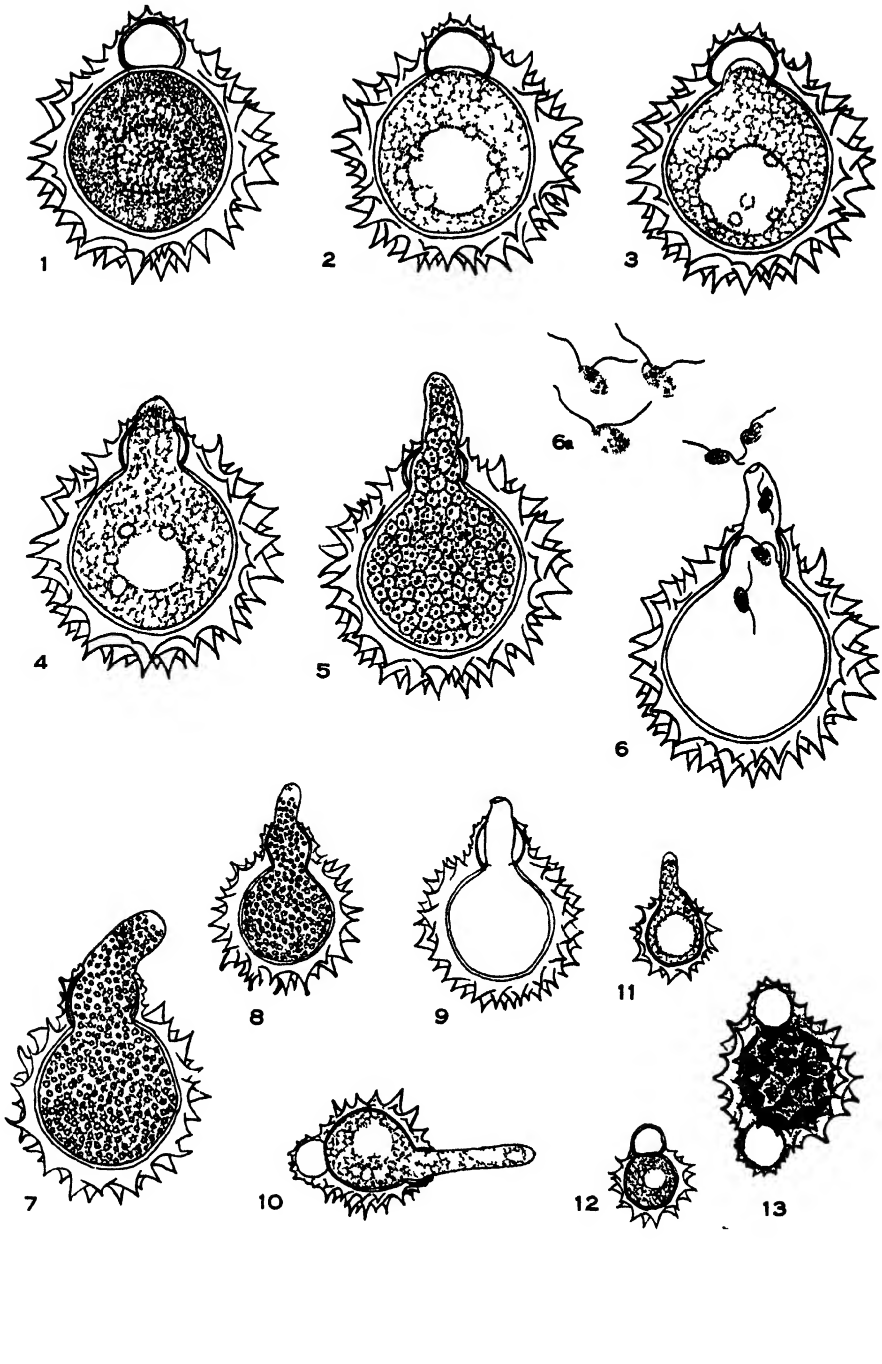
Fig. 9. Empty resting spore showing germination tube not completely filling the companion cell. × 368.

Fig. 10. Medium sized resting spore having two companion cells. Germination is taking place through only one of them. × 368.

Figs. 11 & 12. Two of the very smallest resting spores; one just before the formation of the germination tube, the other farther advanced. × 368.

Fig. 13. Surface view of resting spore accompanied by two companion cells showing ridges connecting spines. Surface view of the companion cells is not shown. × 368.

PLATE 24



STUDIES IN THE GENUS OLPIDIOPSIS. II

The Relationship of *Pseudolpidium* Fischer and *Olpidiopsis* (Cornu) Fischer*

By Leland Shanor

Plate 25 and 3 Text Figures

While collecting for parasites belonging to the genera *Pseudolpidium* Fischer and *Olpidiopsis* (Cornu) Fischer, the author became quite interested in the relationship between them, principally because they are so frequently collected together and because continuous culture has produced only *Olpidiopsis*. This interest was further intensified by the finding in the case of one species of *Olpidiopsis* just described in another paper, instances of clear gradation between what have been considered the zoosporangia of *Olpidiopsis* and what have been thought to be the spiny resting sporangia of *Pseudolpidium*.

In a perusal of the literature relating to the genus *Olpidiopsis* one immediately becomes impressed by the abundance of the citations where members of this genus are found frequently in association with members of the genus *Pseudolpidium*. It is also very interesting to note that the species of *Pseudolpidium* found occurring with a given species of *Olpidiopisis* remains relatively constant when it is collected, even though the stations where the collections have been made are widely separated geographically. It also seemed quite strange that any two parasites so similar in habits should be found occurring together over such a wide range. All of these facts indicated that some very interesting discoveries might be made if the problem were more thoroughly investigated. This study was begun in March, 1938, and has been continued to January, 1939.

Although these parasites were known before, Cornu (1872) was the

* The conclusions reached in this paper concerning *Pseudolpidium Saprolegniae* were briefly stated before the Mycological Society of America, meeting at Richmond, Va., December, 1938, in connection with the variation of sporangial forms of the new species discussed then and described in the first paper of this series. Mr. D. A. McLarty, at the same meeting, read a paper dealing primarily with the validity of *Pseudolpidium fusiforme*.

first to describe and name them as members of the genus *Olpidiopsis*. He observed the zoosporangia to be smooth-walled structures of varying sizes and shapes, and the resting spores to be spiny and thick-walled and to be accompanied by a usually smooth, thin-walled structure which he termed a "cellule adjacente." In one of his species, *Olpidiopsis Aphanomycis*, no resting spores were observed and in another, *O. incrassata*, the adjacent cell was not clearly determined.

In his early studies on *Olpidiopsis*, Fischer (1880, 1882) was unable to find an adjacent cell on the spiny structures which he thought were the resting spores of this genus and concluded that Cornu (1872) had been mistaken in his observations in this respect and that the resting bodies were not accompanied by an adjacent cell. When he later discovered the true resting spores of *Olpidiopsis* that were accompanied by the companion cell as had been described by Cornu (1872), Fischer (1892) concluded that Cornu was correct in his former observations. Fischer now retained the generic name given by Cornu (1872) for those forms whose resting spores were accompanied by the adjacent cell and established the genus *Pseudolpidium* to accommodate those forms which had been studied before in which the companion cell had been lacking. The chief difference in the two genera was, therefore, the presence of this cell on the resting spores of *Olpidiopsis* and the lack of it in *Pseudolpidium*. In other respects the two genera are alike and impossible to distinguish unless the resting spores are present.

In his new genus *Pseudolpidium*, Fischer (1892) placed six species. *Pseudolpidium Saprolegniae* was the name given to the plant studied previously by him and taken in part from Cornu's original *Olpidiopsis Saprolegniae*. *Pseudolpidium fusiforme* was the name applied to the plant which he had thought before was *O. fusiformis* Cornu, and was obtained by splitting Cornu's *O. fusiformis* to form this species of *Pseudolpidium* and *Olpidiopsis minor* n. sp. *Pseudolpidium Aphanomycis* was proposed doubtfully by transferring *O. Aphanomycis* Cornu, the species in which resting spores had not been observed, to this genus. *Pseudolpidium* (?) *incrassata* was also transferred doubtfully since the adjacent cell to the resting spore of *O. incrassata* Cornu had not been clearly shown. *Pseudolpidium glenodinianum* and *P. Sphaeritae*, in spite of the fact that their resting spores were unknown, were placed here by transfer from the genus *Olpidium* to which they had originally been assigned by Dangeard (1888, 1889).

Of the six species which he placed in the genus *Pseudolpidium*, Fischer had the opportunity to study only *P. Saprolegniae* and *P.*

fusiforme in any great detail. By this establishment of *Pseudolpidium* to include the forms lacking a companion cell on the resting spores, Fischer thought that the confusion caused by his previous work had been cleared up.

Those making a survey of the fungi of any certain region are not usually greatly concerned with culturing and studying in detail those forms that have been adequately described before, and so students as a rule accept an original authoritative description when making an identification unless some interesting observation leads to a more detailed study. Therefore, students since Fischer have been inclined to accept unquestionably the large spiny structures lacking a companion cell that are so frequently found associated with certain species of *Olpidiopsis* as the resting spores of *Pseudolpidium*, since the two were said to be commonly found together. Then, too, a determination has too frequently been made and reported as *Pseudolpidium* when only the large smooth-walled sporangia were seen.

MATERIALS AND METHODS

The isolates from which these observations have been made have been collected from various stations in North Carolina, mostly in the vicinity of Chapel Hill. They have all been obtained parasitizing watermolds that have grown on halves of boiled hemp seed which have been placed in water collections when these were brought into the laboratory.

The procedure followed for the isolation and purification of all cultures of *Olpidiopsis* and *Pseudolpidium* was as follows: When an original culture of the watermold was found to have hyphae infected with what appeared to be a species of either of these two genera of parasites, a hypha containing an early infection was carefully removed, washed, and then placed on M. P. #5 agar. In this way pure cultures of the host were easily and quickly obtained. Fresh pure cultures of the host for the isolation of the parasite were then secured by placing halves of sterilized hemp seed in a petri dish in water on small blocks of the agar containing the watermold. When the watermold hyphae began to appear around the edge of the hemp seed, infected hyphae bearing mature sporangia were removed from the original culture, cleaned thoroughly in distilled water, and then transferred to these young pure cultures of the host. Usually a vigorous infection by the parasite is soon obtained in this way. No attempt to get single spore or monosporangial cultures was made at this time, the purpose now being to get the parasite clean and growing vigorously on a single host plant.

As the study progressed and the relationship of smooth and spiny sporangia became more obvious, it was necessary to make monosporangial and single spore isolations. Monosporangial cultures were made in two ways: (1) A hypha bearing a single sporangium of the desired type was cut off from the culture, removed and thoroughly washed in distilled water, examined with the compound microscope for attached spores, and if found free of them, then transferred to a young pure culture of the host plant. (2) Single sporangia, when mature, were dissected out of the host hyphae by carefully splitting open the hyphal wall with a fine, sharp-pointed needle. These were then washed carefully in distilled water, examined under the compound microscope, and transferred by means of a pipette to a young pure culture of the host plant. This second method proved to be considerably more difficult to carry out than did the first, and, when it was determined that the results obtained by this second method were not different from the first, this one was discontinued in the later studies.

Single spore isolations were obtained in the following way: A hypha containing a mature sporangium of the desired type was cut off and removed from the original culture, thoroughly washed in distilled water, transferred to a drop of distilled water on another slide, and then placed under the high power of the binocular microscope so that germination could be followed. When the sporangium germinated, a few spores were drawn into a very fine-mouthed pipette as they emerged from the germ tube. These spores were then placed in another drop of distilled water and still more water added to this so that the spores would be allowed to scatter about more freely. After this a few spores were again drawn into the fine-mouthed pipette and transferred to a young pure culture of the host. It is frequently quite difficult to draw up a single spore to add to a culture of the host, but the number can be reduced to three or four. Even though more than one spore were added to a host culture, it is of no great importance, because spores added to a culture in this way proceed to isolate themselves when bringing about infection on the new culture. Each spore, when it brings about an infection on the host hypha, gives rise to a single thallus of the parasite which retains its identity throughout its development (on this observation see also Barrett, 1912), and, upon reaching its maximum growth, forms a single fruiting structure, usually a sporangium. Therefore, as each thallus from a single spore reached maturity and formed a sporangium, the hypha bearing it was carefully removed, washed, and then transferred to a fresh young culture of the host plant. In this way the progeny

of the spores from this sporangium all came from the infection brought about by a single isolated spore. Single spore isolations were made only to verify the results obtained from monosporangial cultures.

The water used in these cultural experiments was distilled water to which had been added a small amount of animal charcoal. This was then filtered and sterilized before being used.

A CONSIDERATION OF PSEUDOLPIDIUM SPECIES

(A) *Pseudolpidium Saprolegniae* (A. Braun) Fischer

The smooth and spiny sporangia of *Pseudolpidium Saprolegniae* described by Fischer (1892) as the zoosporangia and resting spores respectively, have been found not entirely confined to one single species of *Olpidiopsis*. The author has collected isolates producing both of these structures which have proven to be variations of sporangia of *Olpidiopsis Saprolegniae* Cornu, *O. vexans* Barrett (*O. Saprolegniae* (Cornu) Fischer), and *O. varians* Shanor. Since the smooth-walled zoosporangia of all of these species are much alike in shape and size, and since the shape of the spiny bodies of *Pseudolpidium Saprolegniae* has been described as not unlike that of the zoosporangia, when it was determined with certainty, this fact was not at all surprising. Although these *Pseudolpidium* types have appeared with all of the above mentioned species, detailed studies in this group have been largely confined to one *Olpidiopsis* species, *O. varians*. This species lends itself particularly well to a study of this kind because it quickly produces an abundance of sporangia, and in addition, has another advantage because its resting spores have been germinated. Then too, the spiny sporangia in the other species are much more rare and those of *O. vexans*, strictly speaking, have no spines but only wart-like deposits. Cultural studies with *O. Saprolegniae* Cornu and with *O. vexans* Barrett have been carried on only so far as to determine that the smooth and spiny-walled structures suggestive of *Pseudolpidium* were sporangial forms of these two species.

The first type of sporangia produced by the infection by spores from a monosporangial transfer of *O. varians* is usually of either the large smooth-walled or of the large spiny-walled *Pseudolpidium* type, these commonly appearing singly in a host hypha. In later infections in the same culture, brought about by the spores from the germination of these sporangia, zoosporangia regularly appear much smaller and many more of them are usually found in a single hyphal enlargement. As more sporangia mature in a culture, more spores are present to infect young

hyphae as they are produced by the host, and consequently the later increase in the number of sporangia in an enlargement is easily explained. In infections where a single spore has germinated on a hypha, a greater amount of host protoplasm has been available for the growth of the individual thallus than would be available for the growth of each where many spores have developed in a single hypha. This difference in the amount of available food for the growth in the two instances would explain the difference in the size of the sporangia under the two circumstances. Of course, some of the large sporangia are produced in older cultures along with the smaller ones, but this most frequently occurs where only a very few zoospores have brought about the infection of a hypha.

The length and abundance of the spines on the sporangia vary considerably. They may be small and sparsely scattered, long and scattered, or small or long and densely covering the sporangial wall. In still other cases the spines are reduced to mere warts on the wall and instances have been observed where this deposit was so slight as to be hardly observable. Treatment with chloro-iodide of zinc, however, makes the deposit plainly demonstrable because the sporangial wall itself gives a beautiful blue reaction with this solution while the deposit forming the spines remains colorless. All imaginable gradations from perfectly smooth-walled sporangia to those densely covered with long spines could often be found in a single heavily infected hypha from the progeny of a single spore isolation (Text fig. 1a; Plate 25, fig. 3).

The length of the spines or the abundance of them on any sporangium does not seem necessarily to be restricted to sporangia of any given size. Small sporangia may bear spines or be perfectly smooth-walled, and the same can be said of all sizes, even to the very largest ones. However, small sporangia are more commonly smooth-walled or have only a slight deposit, whereas large sporangia, although very often smooth-walled, more often bear longer and more numerous spines. This large spiny type of sporangium (Text fig. 1b, c, d; Plate 25, fig. 3) is the type that in all probability was misunderstood by Fischer (1892) for the "Dauerspore" when he established his *Pseudolpidium Saprolegniae*. Because sporangia of all types mostly appear singly when isolates are originally collected and also because of the difficulty with which the true resting spores of *Olpidiopsis* may sometimes be obtained (see Barrett, 1912, p. 219), it is not surprising that Fischer misinterpreted these structures as he apparently has done.

In my cultures not all of the spiny sporangia were more or less ellipsodial as is said to be typical for *Pseudolpidium Saprolegniae*, but some are somewhat fusiform and distinctly resemble some figures that have been given for *Pseudolpidium fusiforme* Fischer. The somewhat fusiform sporangia produced in cultures of *O. varians* are, however, generally

TEXT FIGURE 1. (a-e), *O. varians* (a) Typical hyphal swelling caused by this species in which there are the spiny-walled sporangia of the *P. Saprolegniae* resting spore type, smooth-walled sporangia, and two *Olpidiopsis* resting spores × 70 (b) *Pseudolpidium* spiny-walled resting spore type of sporangium seen in surface view. × 200. (c) Large elliptical spiny-walled sporangium of the type that is frequently figured as belonging to *P. fusiforme*. × 95. (d) Spiny sporangium, in median plane, showing the formation of the germination tube × 160. (e) Empty spherical spiny type of sporangium showing germination tube and the associated smooth-walled cell still containing some protoplasm. × 115 (f & g), *O. Saprolegniae*. (f) Typical terminal infection of host hypha by this species. × 70. (A terminal infection swelling similar to this is also typical of *O. vexans*.) (g) Single empty spiny-walled sporangium showing the fine, short spines. × 270. (h) *O. vexans*. Sporangium showing few, irregularly scattered wart-like deposits found on some sporangia of this species. × 225.

larger, broader, and more elliptical, and cause much more of a swelling of the host hyphae than do those appearing alone in cultures of *O. fusiformis*. The spines on sporangia of *O. fusiformis* are also much straighter and more coarse (compare Text fig. 1d and Text fig. 2f).

Still other spiny zoosporangia may be perfectly spherical and be

covered with long slender spines. Often these large spherical sporangia have small, smooth, thin-walled cells associated with them and present the appearance of *Olpidiopsis* resting spores (Text fig. 1e). The protoplasm in these smooth-walled adjacent cells, however, does not pass over and fuse with that in the larger spiny one, but instead disintegrates so that often when the sporangium has germinated, some residue can still be seen in this accompanying cell. This spiny spherical type of sporangium just described is of particular interest because it shows more clearly the morphological relationship in the origin of the ordinary zoosporangia in *Olpidiopsis* to that of the resting spores.

The germination of the *Pseudolpidium* type spiny zoosporangia appears to be identical with that of the smooth-walled ones and the former seem to possess no more resting characteristics than do the latter. This is more clearly brought out when cultures heavily parasitized are allowed to dry. When this is done, the contents of both the smooth-walled and spiny-walled zoosporangia shrink away from the sporangial wall in an identical manner, and within a short time disintegrate, while the protoplasm in the true *Olpidiopsis varians* resting spores remains alive for a long time, and if sufficiently mature can be germinated.

Further proof that the spiny *Pseudolpidium* type resting spores are not resting spores but are, in reality, just sporangial variations of a true *Olpidiopsis* is brought out by the germination of the *Olpidiopsis* resting spores. If the resting spores of *Olpidiopsis varians* that are sufficiently aged are carefully drawn up into a pipette from an old culture and placed in a drop of fresh water, they will germinate almost immediately. If clean young cultures of the host are added to the fresh water in which the germinating resting spores are found, they soon become infected by the zoospores formed by the germination of these structures. This infection will, in time, produce all of the sporangial types of both *Olpidiopsis* and of *Pseudolpidium* and, in addition, the true resting spores of *Olpidiopsis varians*.

The large smooth and spiny-walled structures described as the zoosporangia and resting spores respectively of *P. Saprolegniae* do not therefore belong to a distinct plant, but are merely variations in the sporangial types of a good *Olpidiopsis*. They have appeared most abundantly in my cultures of *O. varians*, but when first observed and described by Fischer (1880, 1892) as belonging to *Pseudolpidium*, they were probably sporangial variations of *Olpidiopsis Saprolegniae* (A. Braun) Cornu. This is thought to be the case because the deposits referred to

as spines seen rarely on the sporangial walls of *O. vexans* Barrett are more wart-like and it is therefore unlikely that it was this species. The terminal hyphal swellings figured by Fischer (1880, Taf. X, fig. 5, 6, 7; 1882, Taf. XIII, figs. 2 & 3) containing the smooth and spiny-walled sporangia are the type produced typically by either *O. Saprolegniae* or by *O. vexans*, but the spines are of the *O. Saprolegniae* type.

Earlier Cienkowski (1855) and Pringsheim (1860) had figured both the smooth and spiny-walled sporangia which appear to be those of *O. Saprolegniae*, but neither author correctly understood the nature of the structures with which he was dealing. Pringsheim figured in addition a resting spore with the companion cell typical of *Olpidiopsis*.

(B) *Pseudolpidium fusiforme* (Cornu) Fischer

Of all the species of *Olpidiopsis* and *Pseudolpidium* that have been reported collected together, *O. minor* and *P. fusiforme* seem to appear the most frequently.

Cornu (1872) originally described the sporangia of his *Olpidiopsis fusiformis* as being most frequently fusiform or linear in shape and as being smooth-walled. The resting spores he described as having broad tapering spines and having a thin, smooth-walled companion cell.

When Fischer (1880) first found the fusiform sporangia of this species, he also found fusiform spiny bodies and, since he did not find the resting bodies as had been described by Cornu, concluded that these must be the resting spores of *O. fusiformis*. After discovering a true member of the genus *Olpidiopsis* later, Fischer (1892) concluded that two plants were involved in Cornu's orignial description of *O. fusiformis*. The one of these Fischer described as having fusiform smooth-walled sporangia and fusiform spiny-walled resting spores, and named it *Pseudolpidium fusiforme* (Cornu) Fischer. This was the plant that he had observed earlier. The other plant Fischer characterized as having small, more spherical or oval smooth-walled sporangia and the type of resting spore as described by Cornu. For this plant Fischer proposed the name *O. minor*. He says, furthermore, that these two forms seem always to appear together and cites to substantiate this statement the observations of Cornu (1872) and of Reinsch (1878) also.

Butler (1907) has reported *O. minor* from India but in his plant the sporangia were described as being larger and similar to those of *P. Saprolegniae*, from which, he says, they could not be distinguished.

Petersen (1910) has obtained *O. minor* and *P. fusiforme* together from Denmark (Sealand and Jutland) but failed to find any of the *Pseudolpid-*

ium fusiforme spiny resting spores. Von Minden (1915) also states that *P. fusiforme* is often found with O. minor.

Sparrow (1932) has collected both *O. minor* and *P. fusiforme* from Cold Spring Harbor. He does not mention their ever appearing together in the same culture but says that some of the zoosporangia of

TEXT FIGURE 2 *Olpidiopsis fusiformis* Cornu (a) Hyphal tip containing five sausage-shaped sporangia of the *Pseudolpidium fusiforme* type × 70 (b) Hyphal tip containing in addition to the *P fusiforme* type, small oval sporangia and resting spores typical of *O minor* × 70 (c) A hyphal enlargement showing fusiform sporangia of the *P fusiforme* type and resting spores of *O minor* type × 70 (d) Swollen hyphal tip showing typical *O minor* infection containing the small sporangia and resting spores × 70 (e) A single *O minor* oval type of resting spore showing the broad tapering spines and the smooth-walled companion cell × 350 (f) Spiny-walled sporangium of the *Pseudolpidium* resting spore type showing scattered prominent spines × 140

O. minor possess spines. Tokunaga (1933) reports them both from Japan and found them frequently occurring together

Matthews (1935) reports *P. fusiforme* and *O. minor* from Mountain Lake, Virginia, both growing on hyphae of the same collection of *Achlya flagellata*. They have appeared together invariably in my cultures, and the spiny-walled *Pseudolpidium* resting spore type were found almost as abundantly as the smooth-walled fusiform sporangia

For the study to determine the relationship of this species, monosporangial and single spore isolations were made in the same manner as was done for the study and determination of *P. Saprolegniae*, and the results have been very similar. The first type of sporangia produced by the spores from a monosporangial transfer from any of the sporangial types are usually the fusiform ones, most commonly the smooth-walled type. If only a few spores infect a hyphal tip, very little if any enlargement takes place, the sporangia are sausage-shaped to fusiform, and if no further cultural studies were made, the immediate diagnosis would be *P. fusiforme* (Text fig. 2a). As these sporangia germinate, however, the infection brought about by these spores produced the typical more abundant, smaller sporangia and resting spores in the swollen hyphal tips characteristic of *O. minor* (Text fig. 2d). In a good many infections the fusiform sporangia, the small, more oval sporangia, and the resting spores of *O. minor* all appear together (Text fig. 2c). In other instances, the hyphal tip may be swollen and contain only fusiform sporangia and the *O. minor* type resting spores (Text fig. 2b).

The resting spores of the isolates of *O. minor* which I have studied are more frequently oval than spherical but have the broad tapering spines characteristic of this species (Text fig. 2e). The spines on the spiny sporangia are not usually as numerous as are those on the spiny-walled sporangia discussed under the preceding species (Text fig. 2f). The interesting gradation between smooth-walled and spiny-walled sporangia is not so clearly evident as it was in the case of *O. varians*. Neither are all of the spiny sporangia fusiform or linear, but spiny sporangia may frequently be oval or nearly spherical as were those reported by Sparrow (1932). The very large spherical spiny sporangia with the functionless adjacent cell described under *O. varians* have not been observed in cultures of *O. minor*.

In the light of these observations, it becomes necessary to recombine *O. minor* Fischer and *P. fusiforme* (Cornu) Fischer under the original name give by Cornu, *Olpidiopsis fusiformis*, since what have been described as the smooth-walled sporangia and spiny-walled resting spores of *P. fusiforme* are merely sporangial variations of the same *Olpidiopsis*.

(C) *Pseudolpidium Aphanomycis* (Cornu) Fischer

The problem involved relating to the validity of *P. Aphanomycis*, as it has developed from my cultures, is not the same as that for the two species just discussed. In the former two, the *Pseudolpidium*

resting spores have turned out to be sporangial variations which had been misinterpreted originally. In the case of this species on *Aphanomyces*, the structures thought at first to be resting spores of *P. Aphanomycis*, upon careful examination, have proven to be inadvantageously oriented resting spores of *Olpidiopsis luxurians* Barrett (Text fig. 3, b & d; Plate 25, fig. 5, 5a). The hyphal enlargements produced by this parasite are usually in a different position on the hypha from those of the other species and are more spherical so that the resting spores are frequently found in such a position that the companion cell does not show. Therefore, at first, many of the resting spores appeared to lack

TEXT FIGURE 3. *Olpidiopsis luxurians*. (a) A terminal hyphal enlargement containing a single germinating sporangium. × 125. (b) Hyphal enlargement with one improperly oriented resting spore appearing as a resting spore of *P. Aphanomycis*. × 500. (c) Warted-walled resting spore of type described by Petersen for *O. Aphanomycis*. × 400. (d) Terminal hyphal enlargement containing a typical *O. luxurians* resting spore. × 400.

this structure and were suspected of belonging to *Pseudolpidium Aphanomycis*. It was only by dissecting these resting spores out, and by placing them in a drop of water so that they could be turned about, that the companion cell could be demonstrated in many instances.

The companion cell to the resting spores of this species on *Aphanomyces* is also so thin-walled that it frequently becomes collapsed in such a manner that its position is often difficult to determine among the spines on the resting spore.

The figure of the resting spore of *Olpidiopsis Alphanomycis* given by Dangeard (1891, Pl. 4, fig. 11) shows no companion cell and has been

considered to be a resting spore of *P. Aphanomycis* by Butler (1907). Butler himself found only one spiny structure in his cultures of *Pseudolpidium Aphanomycis*, but because it germinated so soon after it was formed, he questioned its being a resting spore and felt that what Dangeard had figured was likely the true resting spore of this species. After considerable observation on cultures of *O. luxurians* and a careful examination of Dangeard's figure, it seems likely that what Dangeard has shown was an *Olpidiopsis* resting spore whose companion cell was on the opposite side from that observed, or was a resting spore of *P. Pythii* Butler as is intimated by Minden (1915). Butler's spiny body was undoubtedly a spiny-walled zoosporangium, probably of this species also.

When Cornu (1872) named the species that he found on *Aphanomyces*, he was unable to find the resting spores. He was forced, then, to place this plant in the genus *Olpidiopsis* chiefly on the characteristics of the sporangia, since they were similar to those of the other species studied. Petersen (1910) described the resting spores of *O. Aphanomycis* as being similar to those of *O. Saprolegniae* (Cornu) Fischer, but as being somewhat smaller. This description was based on a single resting spore. Fischer (1892), in the meantime had transferred Cornu's plant doubtfully to *Pseudolpidium*. When Butler (1907) reported *P. Aphanomycis* from India, there was firmly established in the literature these two similar parasites occurring on *Aphanomyces*. Barrett (1912) added a second species to the genus *Olpidiopsis* which parasitizes *Aphanomyces* when he reported *O. luxurians*.

In my cultures of *O. luxurians*, the spines on the resting spores are in some cases reduced to such an extent that they are merely warts, and so might very easily fit Petersen's description of the resting spores of *O. Aphanomycis* (Text fig. 3c). Since this is the case, and if both Petersen and Barrett were dealing with the same plant, it is unfortunate that the single resting spore found by Petersen should have been the uncommon warty type. It is, therefore, quite possible that Cornu (1872), Dangeard (1891), Butler (1907), Petersen (1910), and Barrett (1912) were all working with the same species, and if this be true, it should be considered under the original name given by Cornu, even though the description which he gave was incomplete. Whether this possibility should prove correct may be cleared up by the collection and study of a larger number of isolates of *Olpidiopsis* occurring on *Aphanomyces*.

DISCUSSION

The relationship between certain members of the genus *Pseudolpidium* and members of the genus *Olpidiopsis* is clearly brought out in the foregoing cultural studies. However there still remain several species of the genus *Pseudolpidium* that have not been treated, species that so far have not been collected here. Nevertheless, it would seem in order to review what is known about these and to consider their status since the validity of the genus *Pseudolpidium* is very questionable.

When Fischer (1892) transferred *P. glenodinianum* and *P. Sphaeritae* to *Pseudolpidium* from *Olpidium*, the resting spores were unknown, and the change was based on the characteristics of the sporangia and the zoospores. Since the resting spores are the diagnostic structures separating *Olpidiopsis* and *Pseudolpidium*, these two species might quite possibly be good members of the genus *Olpidiopsis* when their resting spores are discovered. Von Minden (1915) more correctly treats these two species when he includes them questionably in the genus *Pseudolpidium*, placing in this same category *P.* (?) *deformans* Serbinow. It would seem even more correct now to consider these species questionably as belonging to the genus *Olpidiopsis* rather than to *Pseudolpidium.*

Fischer's transfer of *Olpidiopsis incrassata* Cornu to the genus *Pseudolpidium*, even though done doubtfully, seems unjustified, for this species should in all probability remain in the genus *Olpidiopsis* where originally placed by Cornu. Even though Cornu (1872) could not demonstrate clearly a companion cell in all cases, his fig. 12a, shows what seems most certainly to be a companion cell to the resting spore. The structure of the resting spores also indicates a species of *Olpidiopsis.*

It is, therefore, evident that none of the species placed in the genus *Pseudolpidium* by Fischer (1892) can remain there unquestionably. Some must be combined with certain species of *Olpidiopsis*, and those whose resting spores are unknown probably belong in this genus also.

Butler (1907) has described two species of *Pseudolpidium* found parasitizing *Pythium*, *P. Pythii* and *P. gracile*, whose characteristics answer those originally given for this genus. Butler reports that a starved resting spore sometimes persists beside a vigorous spiny one in *P. Pythii* so that these structures simulate the companion cells found in *Olpidiopsis.* He clearly points out the fate of these, showing that they do not function as antheridia as do the companion cells of *Olpidiopsis.* It is hoped that both of these species may be collected soon for they need more critical study. Another species, *P. stellatum* described by Sawada, and translated from the Japanese by Tokunaga (1933), also needs to be

studied more critically before its systematic position can be accepted with certainty.

Of all the species that have been placed in the genus *Pseudolpidium*, the ones that seem the most valid are those described by Butler (1907). Although some combinations of species of *Pseudolpidium* and *Olpidiopsis* have been made in this paper, and a reconsideration of others is suggested, a redescription of the genus *Olpidiopsis* to include the *Pseudolpidium* resting spore type of sporangia will be left to another paper now in preparation, which treats the genus *Olpidiopsis* taxonomically.

SUMMARY

In a monosporangial and single spore isolation study of collections originally appearing to be species of *Pseudolpidium* and *Olpidiopsis*, it has been found that all isolates appearing as *Pseudolpidium* have, by continued cultural study, turned out to be *Olpidiopsis*. The spiny resting spores described for *Pseudolpidium* have been found to be either the spiny zoosporangia or misinterpreted resting spores of the various *Olpidiopsis* species as follows:

a) *Pseudolpidium Saprolegniae* resting spore types have been found occurring abundantly in monosporangial cultures of *O. varians*, and frequently in cultures of *O. Saprolegniae* Cornu, the species to which these *Pseudolpidium* structures likely belonged when described. What might loosely be termed spines on the sporangial walls of *O. vexans* Barrett are merely wart-like deposits. *Pseudolpidium Saprolegniae* is, therefore, not a specific fungus but a combination of sporangial forms of authentic species of *Olpidiopsis*.
b) *Pseudolpidium fusiforme* zoosporangial and resting spore types are only sporangial variations that appear in cultures of *Olpidiopsis fusiformis* Cornu. *Olpidiopsis minor* Fischer is likewise not a distinct species, and must be recombined with *P. fusiforme* under the original name assigned by Cornu.
c) *Pseudolpidium Aphanomycis* resting spores appear to be only inadvantageously oriented *Olpidiopsis luxurians* resting spores or those whose companion cell has collapsed in such a manner that it is difficult to be detected among the spines of the resting spore. The question is also raised as to whether more than one species of *Olpidiopsis* occurs on *Aphanomyces* or if *O. Aphanomycis* and *O. luxurians* are not the same species.

The remaining *Pseudolpidium* species are reviewed and their status discussed. None of the species originally placed in the genus when it

was established can remain unquestionably as *Pseudolpidium* species. The continuance of the genus seems to rest on only two species, *P. Pythii* and *P. gracile*, both described by Butler. Even these two species need further critical study.

A redescription of the genus *Olpidiopsis* to include the spiny sporangial types described as resting spores of certain species of *Pseudolpidium* is necessary, but is being left to another paper.

In conclusion the writer wishes to express his sincere appreciation to Dr. W. C. Coker, under whom this study has been undertaken, for his helpful suggestions and interest in the problem, and to Dr. J. N. Couch for his advice and suggestions on certain phases of the investigation.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

LITERATURE CITED

1. BARRETT, J. T. 1912 Development and Sexuality of some Species of Olpidiopsis, (Cornu) Fisher. Ann. Bot. 26: 209–238.
2. BUTLER, E. J. 1907 An Account of the Genus Pythium and some Chytridiaceae. Memoir of the Dept. of Agric. in India, Bot. Ser., I, No. 5, pp. 1–160.
3. CIENKOWSKI. 1855 Algologische Studien, I. Bot. Zeit. 13: 801–806.
4. CORNU, M. 1872 Monographie des Saprolegniées. Ann. des Sci. Nat. Bot., ser. 5, 15: 1–198.
5. DANGEARD, P. A. 1888 Les Péridiniens et leurs parasites. Journ. de Botanique 2: 126–132, 141–146.
6. ——— 1889 Mémoire sur les Chytridinées. Le Botaniste 1: 39–74.
7. ——— 1891 Recherches histologiques sur les Champignons. Le Botaniste 2: 63–149.
8. FISCHER, A. 1880 Ueber die Stackelkugeln in Saprolegniaschläuchen. Bot. Zeit. 38: 689–96, 705–11, 721–26.
9. ——— 1882 Untersuchungen über die Parasiten der Saprolegnieen. Jahrb. für wiss. Bot. 13: 286–371.
10. ——— 1892 Phycomycetes. Rabenhorst's Kryptogamenflora von Deutschland, I, Vol. 4.
11. MATTHEWS, V. D. 1935 Notes on Some Oomycetes from the Vicinity of Mountain Lake, Giles County, Virginia. Journ. Elisha Mit. Sci. Soc. 51: 306–310.
12. MINDEN, M. VON 1915 Chytridiineae. Kryptogamenflora der Mark Brandenburg 5: 209–422.
13. PETERSEN, H. E. 1910 An account of the Danish fresh-water phycomycetes, with biological and systematic remarks. Ann. Mycol. 8: 494–560.

14. PRINGSHEIM, N. 1860 Beiträge zur Morphologie und Systematik der Algen. IV. Nachträge zur Morphologie der Saprolegnieen. Jahrb. für wiss. Bot. **2**: 205–236.
15. REINSCH, P. 1878 Beobachtungen über einige neue Saprolegnieae, etc. Jahrb. für wiss. Bot. **11**: 283–311.
16. SPARROW, F. K. JR. 1932 Observations on the Aquatic Fungi of Cold Spring Harbor. Mycologia **24**: 268–303.
17. TOKUNAGA, Y. 1933. Studies on the aquatic chytrids of Japan. I. Woroninaceae. Trans. of the Sapporo Nat. Hist. Soc. **13**: 20–28.

EXPLANATION OF PLATE 25

Fig. 1. *O. vexans*. Terminal enlargement of hypha of *Saprolegnia litoralis* containing one resting spore; three smooth-walled sporangia, two of which are germinating; and two sporangia on whose walls are irregularly scattered wart-like deposits, one empty, and the other not yet beginning to germinate. × 123.

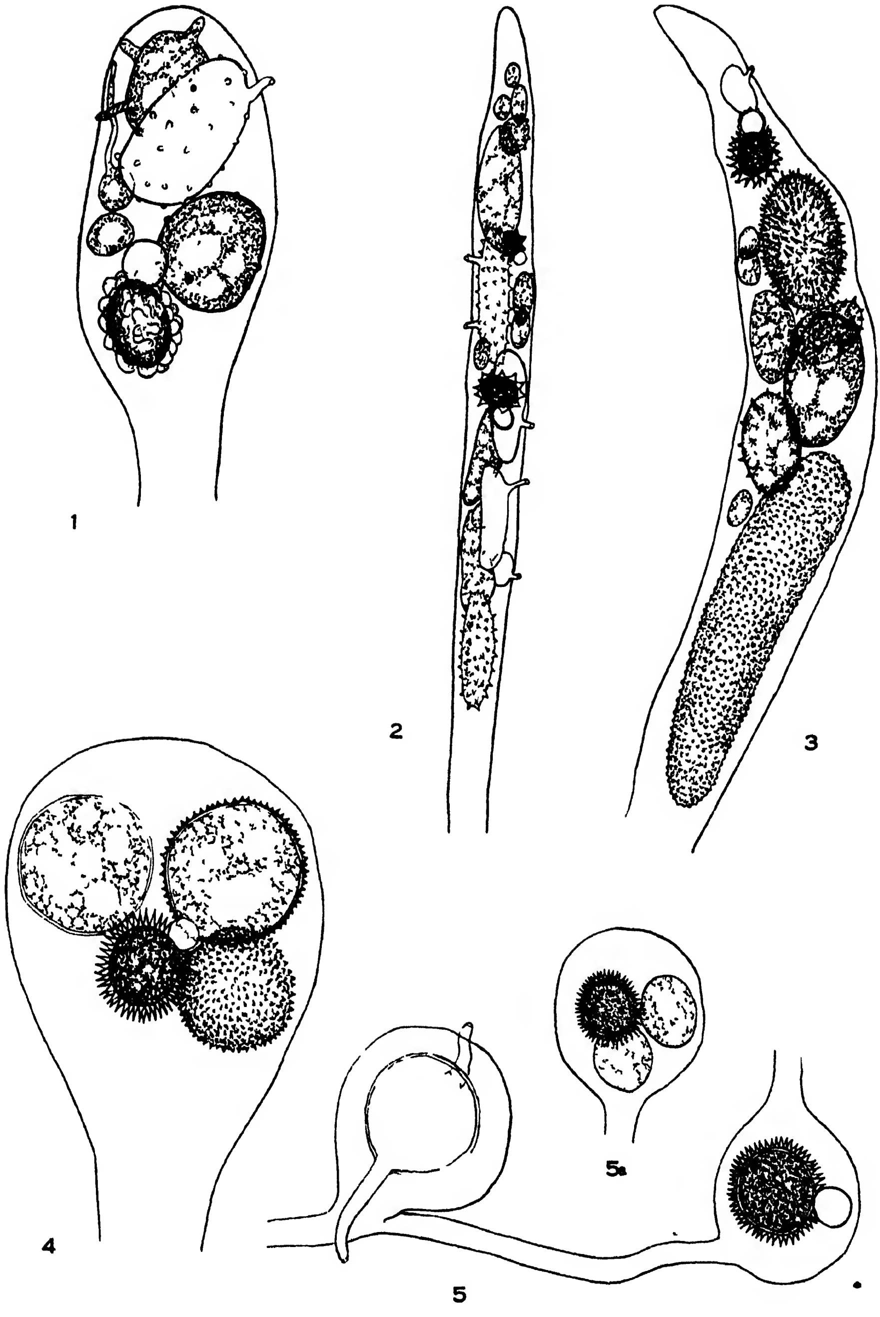
Fig. 2. *O. fusiformis*. Hyphal tip infection in *Achlya imperfecta* showing smooth and spiny-walled fusiform sporangia of the *Pseudolpidium fusiforme* type; small oval smooth and spiny-walled sporangia; and two resting spores. × 123.

Fig. 3. *O. varians*. Terminal swelling of *Achlya flagellata* hypha showing smooth-walled sporangia of various sizes; two sporangia with scattered sharp spines; one very densely spiny sporangium; one very large sporangium whose spines are reduced to small wart-like deposits; and one resting spore. × 123.

Fig. 4. *O. Saprolegniae*. Terminal enlargement of hypha of *Saprolegnia ferax* showing one smooth-walled sporangium; two spiny-walled *Pseudolpidium* resting spore type sporangia, one in median plane and the other in surface view; and one resting spore of this species. × 123.

FIG. 5. *O. luxurians*. Two intercalary swellings of a hypha of *Aphanomyces laevis*, one containing an empty sporangium and the other a typical resting spore of this species. 5a. A terminal swelling of an *Aphanomyces* hypha containing two sporangia and one resting spore. The resting spore is oriented to give the appearance of being a *Pseudolpidium* resting spore since companion cell is on opposite side. × 175.

PLATE 25



NEW MITES FROM WESTERN NORTH CAROLINA

By Arthur Paul Jacot

Plate 26

The following species were obtained from litter samples taken from the Bent Creek Experimental Forest (ten miles south of Asheville on the Brevard road), which were taken to endeavor to determine the extent and rôle of the fauna of litter in causing litter reduction. The types are to be deposited in the U. S. National Museum. Figures of species not here illustrated are in my files and are to appear in a more comprehensive publication.

Cultroribula divergens sp. nov.

Distal end of lamellae directed laterad; anterior edge of rostrum drawn downward in two attenuate cusps with the area between them (as seen in dorsal aspect) thinly sclerotized; anal and genital apertures close together; abdomen quite rotund; tectopedia I well developed, forming a bulge in dorsal outline of sides of cephaloprothorax; size varying in length from 0.23 to 0.24 mm., in breadth from 0.16 to 0.168 mm.

Cotypes: One hundred seven specimens from Andropogon field' herb-moss interspaces between grass plants, Case Place, Bent Creek Exp. Forest; taken February 6th, slide 34F24-8.

No individuals were obtained from the grass clumps.

Cultroribula trifurcatus sp. nov.

Distal end of lamellae directed anteriad though slightly more distant than further back; anterior edge of rostrum drawn forward in three attenuate cusps (trifurcate) as seen in dorso/ventral aspects; anal and genital apertures distant more than half length of genital aperture; abdomen somewhat flattened posterolaterally but quite broad; tectopedia I not forming a bulge in dorsal outline of sides of cephaloprothorax; size smaller and more slender: total length 0.214 to 0.224 mm., breadth 0.128 to 0.14 mm.

Cotypes: four specimens from dogwood (Cornus) litter, thirty-year-old field south of bunk-house, Bent Creek Exp. Forest; taken September 20th, slide 34F4.3-54.

Oppia elongata

Oppia elongata 4, p. 11, I rename *Oppia parviaures* **nom. nov.** since *Dameosoma elogantum* corrected on plate legends to *D. elongatum* 6, p. 43, is an *Oppia.*

Oribotritia virginiensis 3, p. 257, pl. 42

Original figure 77 is correct except that the interrogation points on the aspis should be changed to minute circles to represent the rostral and lamellar bristle insertions. This posterior and lateral position of the rostral bristles is most unusual. In lateral aspect the pseudostigmatic organs look like a black spot because they are viewed end on. The fine combing of the notogaster makes the animal appear iridescent in life and in alcohol. The pocking is faint and appears to be on the inside of the body wall.

I have several specimens from various litter samples from the Bent Creek Exp. Forest.

Euphthiracarus pulchrus pisgahi subsp. nov.

Differs from the species (3, p. 250, pl. 39, fig. 59) in having pseudostigmatic organs somewhat wider but with almost smooth head; anogenital plates pocked over their entire surface.

Cotypes: Six specimens from oak litter, east slope of ridge above Poplar Cove; Bent Creek Exp. Forest; taken July 15th, slide 35F6.2-37.

Phthiracarus apiculatus sp. nov.

Aspis with rostrum projecting beyond rim; carina absent; rostral bristles not unusual; vertex bristles not evident; pseudostigmatic organs oval, eyed, distal end drawn out into a hyaline, sharply pointed, downcurved apicule which is nearly as long as body of organ; notogaster oval, not high, tan to greenish grey (in small specimens), bristles fine, below medium length, depressed, angled proximad of middle, a1 usually near edge of collar; no accessory plate spoon; ventral plate with small, well spaced denticles; genital covers with anterior edge only slightly swollen; anal cover bristles II:1 to II:3 not visible in lateral aspect, I:1 and I:2 widely spaced, I:2 much longer than I:1; diagonal length of notogaster 0.37 to 0.58 mm.

Cotypes: Forty-eight specimens from oak litter, old growth stand, compartment 20, along brook, Bent Creek Exp. Forest; taken August 12th, slide 35F8.2-74.

Phthiracarus setanus sp. nov.

Aspis with very slender, nonprojecting rim; carina absent; rostral bristles not unusual; vertex bristles springing from a definite swelling, arched, just visible in lateral aspect; pseudostigmatic organs long, rodlike, pointed like a lead pencil, bent near pseudostigmata, distal third slightly more swollen than proximal two-thirds; notogaster oval, not high, bristles rather long, the longest about equal to length of genital covers, a1 near edge of collar; ventral plate denticles small; accessory plate without spoon; anterior edge of genital covers barely swollen, the bristles quite conspicuous; anal cover bristles I:1 and I:2 much shorter than II:1 to 00:3, II:2 inserted near transverse plane passing through I:2, II:3 distant from median edge of cover; diagonal length of notogaster 0.5 to 0.65 mm.

Cotypes: Eight specimens from litter of laurel slick, north side of Shut-in-Ridge, east of Walnut Cove, Bent Creek Exp. Forest; taken May 8th, slide 35F1.4-31.

Somewhat resembling *Ph. boresetosus* but bristles shorter; with ventral plate denticles; length of anal cover bristles the reverse; no accessory plate horn.

COCCORCHESTES 5, p. 573

Paul H. Oehser, editor of the U. S. National Museum, has kindly called my attention to *Coccorchestes* T. Thorell 1881 (Ann. Mus. Genova, vol. 17, p. 671, Araneina). I therefore propose as substitute for the generic name of the mite, *Coccorchestes humicolus*, the generic name *Oehserchestes* **nom. nov.**, making *Oehserchestes humicolus* **comb. nov.**

Histiostoma decemvirgae sp. nov.

Abdomen considerably broader and higher than cephaloprothorax; dorsal face with four pairs of conical apophyses each bearing a long, stout, backward curved bristle the length of which is nearly equal to breadth of abdomen, cephaloprothorax with one pair; ventral suckers broad-ovate; tarsi long and slender, tibiae longer than broad (tibiae I twice as long as broad); genuals one and a half times length of tibiae; tibiae with dorsal bristle long and stout, as long as dorsal bristles; sensory club inserted on proximal edge of tarsus, depressed, close to face of tarsus; ventral face with three of the usual spines, one at distal end, one slightly distad of distal fourth, and one at center; hind tarsi longer than tarsi I, bristles similar but with dorsal face bristle at proximal instead of distal fourth; mandibles acicular, palp bristles rather short; total length of adult females 0.2 mm.

Cotypes: Two specimens from litter of old woodland of a thirty-five degree, south slope, Bent Creek Exp. Forest; taken June 17th, slides 35F4.2-1a and -31.

The ten long, stout dorsal bristles, standing high above the body, make this the most easily recognized described American Histiostoma.

Histiostoma tesselata sp. nov.

Figures 1 and 2

Abdomen depressed, posterior end bluntly tapering, sides with flat winglike expanses overhanging the legs, middle area of dorsum areolated, bristles very fine, straight; posterior ventral suckers short, oval, small end anteriad; mouth parts directed forward; rostrum broad, truncate in dorsal aspect; palps large transverse, their bases encompassing the slender mandibles; anterior bristles of palps long, directed upward, posterior bristle quite short; mandibular bristle short, inconspicuous; tarsi I not tapered or constricted, sensory club small, inserted at proximolateral edge of tarsus, not noticeable in lateral aspect; total length of body (including palps) 0.26 mm., of abdomen 0.15 mm., breadth 0.11 mm.

Cotypes: Three specimens from rift of oak litter, 200-year-old woods, Shut-in-Ridge (station 17, compartment 5), Bent Creek Exp. Forest; taken June 11th, slide 35F3X-9.

This lot also included two other specimens on slides 35F3X-7 and -18.

Histiostoma verrucogenicula sp. nov.

Figures 6 to 9

Abdomen broad, rectangular, with an irregular, median hump, the bristles short, bent, depressed; cephaloprothorax much narrower than abdomen, anterior end truncate; mouth parts broad, directed downward so as to be barely visible from above; mandibles acicular, bristle short; anterior bristle of palps long, stout, extending ventrolaterally well beyond legs I; posterior bristle of palps short, strongly recurved; ventral suckers elongate-ovate (quite slender), extending mesad of legs III and IV, the broad end posteriad; leg bristles well developed, sensory club appears to be inserted in a notch cut out of dorsolateral edge of tibiae, thus appearing to spring from distal end of tibiae in lateral aspect; dorsoproximal end of tarsi I with a large wart (excrescence) composed of four or five transverse, crowded ridges; total length of body 0.34 mm., of abdomen 0.2 mm., breadth 0.18 mm.

Cotypes: Four specimens from mull litter of Rocky Cove, Bent Creek Exp. Forest; taken September 8th, 1934, slide 34F1-4.

Two immatures (?) from Liriodendron litter of a south cove, Shut-in-Ridge (B.C.E.F.); taken June 17th, slides 35F4X-37, 35F4.2-28.

Histiostoma verruca sp. nov.

Figures 4 and 5

Length of females 0.25 to 0.29 mm., of males 0.18 to 0.2 mm.; skin granular; body oblong, with posterior bulge, dorsoposterior face of abdomen with a fairly large excrescence, oval in dorsal aspect; bristles short, nearly straight, tapering rapidly; ventral suckers elongate, one between legs II and III, one mesad of legs IV, parallel to median plane; sensory club inserted on posterior edge of tarsus; leg bristles short, except distal which is slightly over half the length of tarsus; mandibles needlelike (unhooked), untoothed, bristle long, curving well beyond sides of rostrum; palp bristles long, fairly stout; males with posterior end of abdomen produced as two prominent, clear edged, semicircular lobes (recalling Histiogaster when seen in dorso-ventral aspects) the posterior edge of which bears a minute bristle inserted laterad of center of lobe; the four suckers close to median plane, oval, the anterior pair separated from the posterior pair by their own length.

Cotypes: Two females from litter of old woodland of a thirty-five degree, south slope, Bent Creek Exp. Forest; taken June 17th, slide 35F4.1-2.

Most closely related to the European *H. fimetarius* as figured by Berlese (1, fasc. 9:7), but easily recognized by the rather large median pea-wart on posterior end of its back.

Although the commonest litter Histiostoma of this region it is uncommon. With the cotypes (but mounted on different slides) were fourteen additional females and three males. Another lot from a northern cove (Rocky Cove), contained three females and one male.

Macrocheles (Coprholaspis) appalachicus sp. nov.

Bristles of dorsum numerous, fine, overlapping, more numerous behind, no particular shoulder bristles; hypostome with a prominent, stout median spine, anterior edge finely sawtoothed to smooth; sternal, genital and anoventral scutes faintly, coarsely areolate, the areolations on sternal scute usually forming concentric semiovals culminating in the "linea oblique anteriores," the anterior ends of which are as approximate as they are distant from the ingular bristles; ingular and metasternal scutes fused to sternal; epigynal scute broad; anoventral scute in males fused to sternal and to peritremal, in females distinct, extending laterad as far as does the peritremal; no repugnatorial pores; no porose (punctate) areas; sculpturing of anogenital scute resembling fish scales; tarsi I retain a diminutive ambulacrum with the usual two nails, half the length of terminal bristles; tarsi II of females with seven stout spinelike bristles (five ventral, two dorsal); distal end of horn of male femur II three-ridged; femora IV unarmed; proximoventral edge of tarsi, tibiae and genuals denticulate; size variable: total length of dorsal shield of females 1.3 to 1.65 mm., breadth 0.8 to 1.0 mm.

Cotypes: Two males and a female from deciduous litter west side of ridge above Poplar Cove, Bent Creek Exp. Forest; taken July 29th, slide 35F7.1-25.

Most closely related to *M. bihastatus* 2, p. 286, fig. 1, differing in absence of the two lateral teeth, in absence of shoulder bristles, the greater armature of female tarsus, and the much greater size.

Twenty-four other adults from ten lots of forest litter of various types about the Bent Creek area are before me, never more than four per square foot. This species may thus be regarded as thinly sprinkled about the forest floor of this region.

NORTHEASTERN FOREST EXPERIMENT STATION,
NEW HAVEN, CONNECTICUT.

LITERATURE CITED

1. BERLESE, ANTONIO, 1882–1900, Acari, Myriapoda et Scorpiones hucusque in Italia reperta. Padova. 101 fascicles.
2. EWING, H. E., 1920, New Predaceous and Parasitic Mites of the Superfamily Gamasoidea (Acarina). Ent. News **31**: 286–293.
3. JACOT, A. P., 1930 (Nov.), Oribatid Mites of the Subfamily Phthiracarinae of the Northeastern United States. Proc. Boston Soc. Nat. Hist. **39**: 209–291, pls. 33–42.
4. ———, 1938 (Jan.), Some New Western North Carolina Moss-Mites. Proc. Ent. Soc. Wash. **40**: 10–15.
5. ———, 1938 (Nov.), Four New Arthropods from New England. Amer. Midl. Nat. **20**: 571–574, 3 figs.
6. PAOLI, GUIDO, 1908 (Sept. 16), Monografia del Genere Dameosoma Berl. e Generi affini. Redia **5**: 31–91, pls. 3–5.

PLATE 26

Histiostoma tesselata sp. nov.

Fig. 1. Lateral aspect of cephalon and leg I; ×500±
Fig. 2. Dorsal aspect of cephalon; ×500±
Fig. 3. Dorsal aspect of abdomen (sketch); ×318±

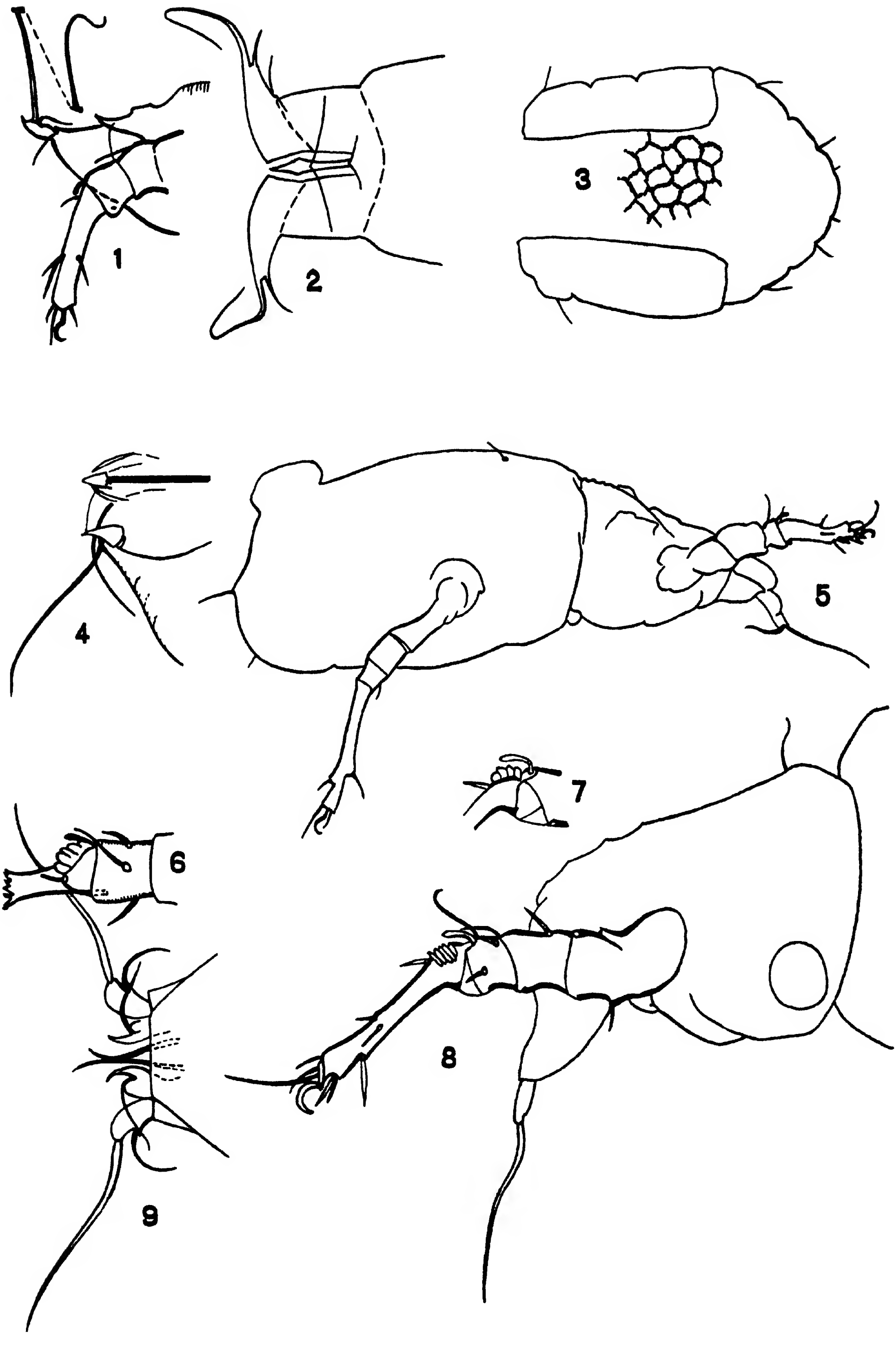
Histiostoma verruca sp. nov.

Fig. 4. Ventral aspect of mouth parts (right half); ×668±
Fig. 5. Lateral aspect, conspicuous bristles only; ×318±

Histiostoma verrucogenicula sp. nov.

Fig. 6. Dorsal aspect of right tibia I, showing wart and clavate bristle; ×500±
Fig. 7. Mesal aspect of same; same mag.
Fig. 8. Lateral aspect of cephalon and leg I; ×500±
Fig. 9. Dorsal aspect of mouth parts; ×500±

PLATE 26



THE ADMINISTRATION OF LARGE DOSES OF ASCORBIC ACID AND OF METHIONINE TO THE CYSTINURIC*

By James C. Andrews and Kathleen C. Andrews

The effect of administration of methionine on the cystine output of the cystinuric subject has been reported during past years by several investigators with somewhat divergent results. Brand, Cahill, and Harris (1) have reported a marked increase in daily cystine excretion while Andrews and Randall (2), working with a preadolescent cystinuric, found that the administration of the same amounts of methionine as employed by Brand and co-workers produced very little extra cystine. Later experiments by Brand (private communication) on a younger (preadolescent) sister of his former cystinuric subject showed no appreciable increase in cystine output. Lewis, Brown, and White (3) reported, from a similar experiment, an increase in cystine excretion intermediate between the results of Brand and co-workers and those of Andrews and Randall. A recent report by Hess and Sullivan (4) indicates that in their hands the feeding of methionine to a cystinuric produced no increase in cystine excretion.

Since cystinuria is essentially a defect in some biological oxidative mechanism, it appeared possible that the positive results obtained in some cases with methionine, cysteine, etc., might be due to the introduction into the organism of large amounts of a reducing substance. The ease with which methionine is demethylated makes its use practically equivalent to that of a corresponding amount of homocysteine. Hence the administration, instead, of large doses of ascorbic acid (Vitamin C) appeared to be a means of testing the hypothesis. Moreover, the fact that we have yet available the same cystinuric subject (now aged 16) as that used three years earlier by Andrews and Randall (2) makes a repetition of methionine administration a matter of interest.

Table I shows the results in terms of 24-hour output obtained from an experiment extending over about 5 weeks. During this time the subject was on a rigidly controlled diet as indicated by the total nitrogen figures.

* An abstract of this paper was published in the Proceedings of the American Society of Biological Chemists, March, 1938.

TABLE I

Effect of administration of cystine, methionine and ascorbic acid on the urinary output of cystine in the cystinuric

DATE	VOL.	TOTAL N	CREATININE	TOTAL S	SO_4—S	PER CENT OXIDATION	CYSTINE S	CuCl-S	α x VOL.	ASCORBIC ACID
	ml.	*gm.*	*gm.*	*gm.*	*gm.*		*gm.*	*gm.*	*ml.*	*mg.*
4/8	920	7.12	0.80	0.457	0.254	55.6	0.125		247	0.6
4/9	790	7.00	0.76	0.416	0.251	60.3	0.110		273	0.8
4/10[1]	850	7.44	0.79	1.248	0.946	75.8	0.117		302	0.8
4/11	1,280	7.32	0.88	0.622	0.444	71.4	0.131		447	1.1
4/12	820	6.91	0.79	0.577	0.400	69.4	0.122		221	0.6
4/13	870	6.94	0.74	0.425	0.267	62.8	0.120		201	0.5
4/14	850	7.00	0.74	0.492	0.287	58.3	0.108		175	0.4
4/15	960	7.26	0.76	0.450	0.296	65.8	0.126		290	0.6
4/16	700	6.86	0.78	0.446	0.275	61.7	0.111		300	0.6
4/17[2]	730	6.97	0.79	0.426	0.261	61.3	0.127	0.150	337	0.7
4/18[3]	3,660	8.45	0.87	0.640	0.398	62.2	0.051	0.201	650	690.0
4/19	1,200	7.02	0.83	0.498	0.298	59.8	0	0.180	480	1,428.0
4/20	870	6.98	0.80	0.443	0.261	58.9	0.079	0.132	391	62.2
4/21	840	7.18	0.83	0.472	0.284	60.2	0.116	0.143	352	13.0
4/22	1,000	7.02	0.74	0.426	0.254	59.6	0.128	0.144	300	1.2
4/23	880	6.94	0.78	0.473	0.289	61.1	0.124	0.148	354	0.6
4/24	800	6.98	0.77	0.446	0.273	61.2	0.131	0.149	377	0.8
4/25[4]	2,520	7.72	0.94	0.590	0.333	56.4	0.182	0.218	630	2.3
4/26[4]	3,920	8.38	0.97	0.601	0.341	56.7	0.197	0.240	676	2.3
4/27	700	6.40	0.72	0.391	0.246	62.9	0.114	0.144	287	1.2
4/28	1,060	6.74	0.77	0.385	0.227	59.0	0.118	0.142	350	1.9
4/29	1,200	6.95	0.79	0.384	0.232	60.5	0.124	0.145	300	0.6
4/30	850	6.91	0.79	0.403	0.241	59.8	0.116	0.151	383	0.5
5/1[5]	890	7.02	0.82	0.400	0.248	62.0	0.048	0.147	395	1.2
5/2[6]	980	6.88	0.78	0.435	0.254	58.4	0	0.163	364	871.0
5/3	1,000	7.16	0.85	0.452	0.279	61.7	0	0.165	330	2,380.0
5/4	800	7.10	0.81	0.399	0.237	59.4	0.040	0.142	316	44.0
5/5	900	6.93	0.78	0.391	0.239	61.1	0.065	0.140	310	30.1
5/6	860	7.01	0.83	0.388	0.243	62.6	0.116	0.131	296	39.5
5/7	890	6.90	0.76	0.425	0.252	59.3	0.128	0.150	312	22.8
5/8[7]	810	7.08	0.78	0.423	0.264	62.4	0.114	0.142	320	0.8
5/9[8]	930	7.22	0.79	0.692	0.488	70.5	0.13	0.168	373	1.2
5/10	990	7.36	0.77	1.302	0.970	74.3	0.156	0.192	445	1.3
5/11	840	7.18	0.73	0.578	0.385	66.6	0.106	0.151	358	0.9
5/12	800	6.92	0.78	0.431	0.283	65.6	0.120	0.144	312	1.1
5/13	940	7.03	0.81	0.412	0.249	60.5	0.104	0.138	342	1.3

[1] 5 gm. cystine fed, 8 A.M.
[2] Ascorbic acid fed: 1 gm. at 8 A.M. and 1 gm. at 1 P.M.
[3] Ascorbic acid fed: 1 gm. at 8 A.M., 1 gm. at 1 P.M. and 1 gm. at 7 P.M.
[4] Water drinking for production of diuresis.
[5] Ascorbic acid fed: 2 gm. at 8 A.M., 2 gm. at 1 P.M. and 1 gm. at 7 P.M.
[6] Ascorbic acid fed: 2 gm. at 8 A.M., 2 gm. at 1 P.M. and 2 gm. at 7 P.M.
[7] 2 gm. dl-methionine fed, 7 A.M.
[8] 5 gm. dl-methionine fed, 7 A.M.

A preliminary administration of 5 gm. cystine (April 10) was used to demonstrate again the extent of conversion of cystine to inorganic sulfate. After several days, ascorbic acid was administered by mouth, 2 gm. on April 17 and 3 gm. on April 18. This dosage seemingly produced excessive thirst with the result that the large volume of urine excreted on April 18 was probably more responsible for any increases than was the ascorbic acid. To ascertain this a water drinking experiment was later inserted (April 25 and April 26) while on subsequent administration of ascorbic acid the water intake was controlled. On May 1 and May 2 there were fed, respectively, 5 and 6 gm. ascorbic acid. These doses were divided into three parts and fed before each of the three meals. On May 8 and May 9, 2 and 5 gm. methionine, respectively, were fed.

Determinations of the usual urinary constituents were made as described in the paper by Andrews and Randall (2). Cystine was determined by the revised Sullivan method as described by Sullivan and Hess (5). However, the large amounts of ascorbic acid excreted after its administration made Sullivan determinations impossible, even by the modified procedure, and it was therefore found necessary to resort to the cuprous mercaptid separation of Russouw and Wilken-Jorden (6). The figures for cystine sulfur obtained by this method are listed under the column headed "CuCl-S". The results of these attempts to apply the Sullivan method to urines high in ascorbic acid confirm our previous results (7) on the inhibitory effect of ascorbic acid on the production of color in the Sullivan method. Even prolonged aeration of these urines, while it reduced the ascorbic acid content as indicated by indophenol titration, did not make possible the development of the Sullivan color. This supports our previous findings as to the presence of reducing agents other than ascorbic acid, which interfere with the Sullivan method, in urine.

The column headed "α x ml." provides further evidence as to the excretion of cystine. Since the optical activity of a cystinuric urine is largely the result of its cystine content, an arbitrary measure of this is provided by the product of the 24-hour volume of urine and the observed activity (α). In this case all readings were made in a 2 dm. tube. This product, while an arbitrary number, serves as a means of detecting marked changes in the total cystine output.

Ascorbic acid was determined by standard indophenol titration as described by Bennett and Tarbert (8).

DISCUSSION

Taking the average value for the daily output of cystine sulfur under normal conditions as 0.120 gm. the effect of methionine administration was to raise this to a maximum of 0.156 gm., a 30% increase. Based on the daily output of "CuCl-S" as determined by the method of Russouw and Wilken-Jorden, an increase from a daily average of 0.142 gm. to a maximum of 0.192 gm., a 35% increase is indicated. This result approaches more nearly that obtained by Brand and co-workers than was the case when our subject was preadolescent but the increase in cystine output is still far less marked than that reported by them.

It should furthermore be emphasized that the diuresis experiment of April 25 and April 26 produced a greater increase in cystine output (47% increase by the Sullivan method and 69% by the cuprous chloride method) than that which resulted from methionine administration. Administration of large doses of ascorbic acid produced, in analyses made by the cuprous chloride method, a maximum increase of only about 16% where the water intake was controlled (May 1 to May 3) in contrast to that of 41% when uncontrolled water drinking was permitted.

The order of increase indicated by the product of "α x ml." leads to the same conclusion. The diuresis experiment produced the largest increase in this product (over 100%). Methionine administration produced an increase of about 50% and ascorbic acid about 30%.

The daily urine volume of the subject is obviously a matter of prime importance in the interpretation of such experiments.

CONCLUSIONS

A comparison was made of the effect of diuresis, of methionine administration, and of ascorbic acid administration on the cystine output of a cystinuric subject.

Doses of ascorbic acid up to 6.0 gm. per day produced, with controlled water intake, an increase of only about 16% over the average normal figure.

Doses of methionine, up to 5.0 gm. per day produced a 30 to 35% increase in the cystine output over the normal. This is a considerably greater increase than that obtained three years previously with the same subject when preadolescent.

Water drinking, approximately quadrupling the daily urine volume, increased the cystine output by nearly 50% by the direct Sullivan method and nearly 70% by the cuprous chloride method.

ACKNOWLEDGMENT

The work herein described was carried on in part in the laboratories of the Medical School of the University of Pennsylvania, Philadelphia. Special thanks are due to Dr. C. B. Rutenber for assistance in making numerous analyses.

UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

BIBLIOGRAPHY

1. BRAND, E., CAHILL, G. F., AND HARRIS, M. M. J. Biol. Chem. **109**: 69. 1935.
2. ANDREWS, J. C., AND RANDALL, A. J. Clin. Investigation **14**: 517. 1935.
3. LEWIS, H. B., BROWN, B. H., AND WHITE, F. R. J. Biol. Chem. **114**: 171. 1936.
4. HESS, W. C., AND SULLIVAN, M. X. Proc. Am. Soc. Biol. Chem. **9**: IV. 1938.
5. SULLIVAN, M. X., AND HESS, W. C. J. Biol. Chem. **116**: 221. 1936.
6. RUSSOUW, S. D., AND WILKEN-JORDEN, T. J. Onderstepoort J. of Vet. Sci. and Animal Industry **4**: 453. 1935.
7. ANDREWS, J. C., AND ANDREWS, K. C. J. Biol. Chem. **118**: 555. 1937.
8. BENNETT, A. H., AND TARBERT, D. J. Biochem. J. **27**: 1294. 1933.

TECHNIC FOR COLLECTION, ISOLATION AND CULTURE OF CHYTRIDS

By John N. Couch

By applying the collecting methods used for water molds (Coker, 1923; Harvey, 1925) we have been able to collect numerous Chytridiales and related forms from water and soil. The water with decaying leaves, grass, trash, and mud is put in Petri dishes or other suitable containers and to the soil collections is added charcoal water (used because the spores of aquatic Phycomycetes are very sensitive to distilled H_2O). Charcoal water (Couch, 1931) is prepared as follows: 1 litre distilled H_2O and 1 level teaspoonful of blood or animal charcoal; filter after 1 hour; autoclave. For bait, pollen grains (Zopf, 1887) (*Liquidambar* is particularly good and may be kept dried for years) or boiled pieces of young corn leaves or grass leaves have been used (Karling 1937, 1938, and numerous earlier papers). The pollen grains are picked up on a needle and dusted on the surface of the water; the pieces of leaves are merely dropped in. After being in the dish two or three days, the bait should be examined. If, on first examination, there are no chytrids, fresh leaves should be added, for some chytrids may be slow in appearing. Decolorizing the leaves by boiling in 95% alcohol in a water bath (a process introduced into our laboratory by Miss Berdan, summer 1938) facilitates the study of the chytrids, but such leaves are not so good as those with chlorophyll for the growth and preservation of chytrids. Other chytrids occur on algae and the oogonia and threads of various filamentous aquatic Phycomycetes.

Since only a few chytrids (Zopf 1887, Sparrow 1931, Butler and Humphries 1932, Karling 1937, 1938, etc.) have been isolated in pure fungal culture and none in single spore or single sporangial culture, it seemed worthwhile to try to develop methods for their isolation and culture free from other fungi and if possible free from bacteria. This paper is a preliminary report on the methods used, with a list of the chytrids so isolated and in culture. For the sake of brevity the methods used may be illustrated by chytrids found on pieces of boiled grass leaves.

After one has discovered the presence of chytrids on a piece of grass in the original dish, the piece is transferred to a sterile Petri dish and washed thoroughly with a strong stream of water from a wash bottle, holding the leaf meanwhile with forceps and tilting the dish so that the trash and many of the undesirable organisms are removed.

Sometimes one may secure a single chytrid species on one piece of grass but more frequently several species occur, as well as leaf-inhabiting forms of *Pythium* and other water molds.

Changing the water will hasten spore discharge of the chytrids as well as the other fungi, hence it is well to have culture dishes, tools, binocular microscope, and everything needed ready before the leaf is removed from the original dish. An examination with the compound microscope (low power) will show what material is worth isolating and enable one to slash off and discard large areas of the leaf infected with *Pythium*, etc.

We have found the following methods most effective in isolating chytrids in pure fungal culture, i.e. free from all other organisms except bacteria and in some cases free from bacteria: (1) isolation in water of a single sporangium or (2) isolation on agar of a single sporangium; (3) isolation of spores from a single sporangium on slide; (4) isolation of single spore in a capillary tube or (5) isolation of single spore on agar or (6) isolation of a single thread or several threads on agar.

With method (1) the procedure may be as follows: Place leaf with chytrids in a drop of water in a Petri dish and in another drop of water about one cm. away in the same dish put a new piece of sterile leaf. Then under a wide field binocular dissecting microscope (×40–×100) with sharp, smooth, steel needles dissect out a small piece of leaf to which only one sporangium is attached. Transfer this to a small drop of water in the same dish and examine under compound microscope to be sure only one sporangium and no spores are present. The fragment of leaf with the sporangium may now be transferred to the fresh piece of leaf. All transfers up to this stage are made from drop to drop in the same dish, because if the delicate chytrid were transferred from one dish to another, it might dry up in the operation. After the chytrid sporangium is on the large, moist leaf, the latter is transferred to a drop of charcoal water in a fresh sterile dish and other drops of water are added to the floor and ceiling of the dish to prevent desiccation. In this operation many of the single sporangia are injured in transfer. Hence it is advisable to make large numbers of transfers.

If one is lucky, each isolated sporangium will form zoospores which

will infect the new leaf, thus establishing the chytrid in pure culture save for the presence of bacteria.

The above technic is useful only if the sporangia are large. A more useful technic (2), particularly with small sporangia, and where several species are mixed and are discharging spores simultaneously, is as follows. An infected leaf is transferred after washing to the surface of a 3% agar plate (see formula below). The desired chytrid sporangium is now dissected out from the leaf tissue and dragged along on the surface of the agar to free it from spores, bacteria, etc. After examining under the compound microscope to make sure that only one chytrid sporangium is now present, it is cut out with a little cube of agar and transferred to a fresh Petri dish in a drop of water with a fresh piece of leaf, other drops of water being added to the bottom of the dish to prevent desiccation. This is a very useful technic because it enables one to transfer nothing but the sporangium and its rhizoids. We have used a slight modification of this method by tearing the leaf tissue apart on the surface of the agar and spreading it out. If water is present, some of the sporangia may discharge their spores on the surface of the agar. The spores may then germinate in contact with the leaf, sending their rhizoids into the agar. It is possible then to remove the leaf, wash the surface of the agar with water from the wash bottle and then to dissect out one sporangium from the agar surface.

If the rhizoidal system is very complex and the spores of other fungi abundant, the method just described may be unsatisfactory, in which case the following method (3) is useful. A single sporangium about ready to discharge spores is isolated by method 1 or 2 and put on a sterile slide in a drop of water and kept under observation so that one may determine just when the spores emerge. The moment this happens some of the spores are drawn up in a capillary tube and blown out in a drop of water with a piece of sterile leaf in a fresh Petri dish. If ordinary care is used one may secure cultures by this technic descended from a few spores. It is possible so to perfect this method that one can, with a little practice and skill, make single spore cultures. This may be done as follows: (4) By using a very fine capillary tube and picking up only a few spores, then diluting the spores by mixing in another drop of water and so on by further dilutions, it is possible finally to get only one or two spores in the capillary tube. If the tube were clean to begin with, it may be examined on the surface of the Petri dish to determine how many spores it contains. If only one spore is present, the tube is then transfered to a drop of water containing a piece of leaf. If it

contains two or even several spores, it is possible to break the tube in such a way that one can separate a single spore from the others.

None of the methods so far described separates the chytrids from bacteria, although it is possible with great precaution to keep the cultures relatively free from them. It is possible, however, to isolate some of the chytrids in pure culture. So far we have developed two methods for doing this. The easier, if the spores will germinate on agar, is by the isolation of a single spore on agar (5). After much experimenting with spore germination on nutrient agars, we have found several which are useful in this work.

1. Plain agar 3% (the agar shreds should be washed over night in several changes of water to free from trash).

2. Agar No. 12 (Leitner's agar) 2% agar and 0.004% peptone (meat).

3. Agar No. 13 (Foust's agar) 2% agar and 0.15% maltose and 0.004% peptone (meat).

4. Corn meal agar (use 2–4 heaping teaspoons full to 1 litre water, depending upon strength desired. Heat gently in water bath, temperature about 60°, 1 hour. Filter. Add water to make 1 litre. Agar 2%).

The spores seem to germinate better on plain agar or agar with very small amounts of nutrient material. Before taking time to spread the spores carefully on agar it is worth while to drop a few on the medium used to determine whether or not the spores go to pieces or settle down and encyst. Naturally if the spores are plasmolized by the nutrient agar it is a waste of time to go further with that particular medium. Spores to be isolated should be as free from bacteria as possible. Hence, it is well to isolate one or a few sporangia about to discharge spores in a drop of water on a sterile slide. However, where only a few spores are available it is possible to pick them up with a platinum loop or a small pipette directly from the original dish. The essential part of this technic is to spread the spores so well on the surface of the agar that some of the spores will be completely separated from the bacteria and other organisms. A successful spreading requires a firm agar (2–3% agar) and a steady hand. The spores are picked up with a platinum loop and the loop dragged along the surface of the plate in a straight line. Several east-west lines may be made and then another group of north-south ones. It is unnecessary to mark these lines for the bacterial colonies will make the lines quite evident. If the laboratory is clean and free from spores of *Penicillium*, etc., it is possible to do this spreading with the uncovered dish on the table. However, if the air is dusty, the dish should be held at an angle with the agar surface down. If the

spores germinate at all they will germinate within 12 to 24 hours or in even less time. After 12 hours the plate should be examined under the binocular dissecting microscope (×40–×100). If the spores have been properly spread, one may now cut out a tiny block of agar with a single

TABLE I

NAME	DATE	FOUND ON	ISOLATED BY	CULTURED ON	PRESENT CONDITION
Rhizidiomyces apophysatus	1932	Ao	1, 2, 5	13, Ao, p.	pfc
Catenaria-like without rhizoids	1932	p	1, 5	p, Ao, 13 etc.	pc
Rozella septigena	1938	A. c.	Host threads and par. spores	Host	pfc + host
Woronina polycistis	1938	A. f.	Host threads and par. spores	Host	pc + host; pfc + host
Rhizophidium carpophilum	1934	Ao	5	h, 13, p, cl	pfc
Rhizophidium multiporum	1937	Ao	5	h, 13, p, cl	pfc
Rhizophidium n. sp.	1937	Ao	5	h, 13, p, cl	pfc
Rhizophlyctis rosea	1938	gl in soil collection	1, 5	cn, cl, gl, fp	pfc
Rhizophlyctis n. sp.	1938	gl in soil collection	1, 2	cl, gl, fp	pfc
Nowakowskiella elegans	1938	gl in soil collection	2	cl, gl, fp	pfc
Siphonochytrium n. gen.	1938	gl in soil collection	4, 5	13, gl, cl, fp	pfc
Cladochytrium replicatum	1938	gl in H_2O collection	6	Various agars	pc
Lagenidium giganteum	1933	Mosquito larva	5	Various agars	pc
Myzocytium sp.	1938	Closterium	2	Various agars	pc
Resticularia (?)	1938	Oscillatoria	6	Various agars	pc

sporangium descended from a single spore. The cutting out operation requires a very fine tool. For this we use a tiny chisel made by sharpening down a steel needle under the microscope. Individual sporangia may be transferred to a drop of water in a sterile Petri dish on a piece of

leaf. Such a culture descended from a single spore may be kept free from bacteria for a few generations in water cultures. In some chytrids the sporangia mature and discharge their spores on agar. It is possible, though exceedingly tedious, to keep such cultures pure, growing on agar and free from bacteria. The labor involved, however, is excessive and we have carried such cultures on agar for only a few generations.

In some of the polycentric chytrids as *Cladochytrium replicatum* the spores germinated on agar to produce a distinct mycelium. It is therefore possible to isolate this species by cutting out a single thread or several threads. This method (6), however, is useless with the monocentric chytrids.

The following table summarizes some of our results in trying to culture chytrids and related forms. In each case the name of the chytrid is given, the host or substratum on which it was found, the method, 1–6 (see p. 209 above), by which it was isolated, the substrata used in its culture and its present condition. The agars used are referred to by letters or number, i.e. agar no. 12, 12; agar no. 13, 13; and corn meal agar, cm. Other substrata or hosts are also abbreviated: oogonia of *Achlya*, Ao; *Achlya caroliniana*, A. c.; *Achlya flagellata*, A. f.; pollen grains, p; boiled corn leaves, cl; boiled grass leaves, gl; boiled filter paper, fp. In the last column the present condition of the fungus is indicated: Pc, pure culture free from other organisms; pfc, pure culture of fungus but with bacteria. Only the species now in culture are listed in table I.

SUMMARY

Methods are described by which 15 representative species from all the larger groups of the Chytridiales and related forms have been isolated and cultured, some for as long as seven years. All of these forms have been isolated by a single sporangium, or a single spore, or pure threads and hence the cultures descended from such isolations are known to consist of a single fungal strain. Several forms are in pure culture on agar, the others are in single fungal culture but with bacteria. In addition to the usual substrata as boiled leaves and pollen, four different species (see table fp) have been grown on boiled filter paper in water. In such cultures bacteria have been present. Indeed, bacteria seem to be necessary for their growth, since spores put on sterile filter paper in water failed to grow. Four species of monocentric chytrids, *Rhizidiomyces apophysatus*, *Rhizophidium carpophilum*, *R. multiporum* and *R.* n. sp. have been carried on agar in pure culture

through several generations. This is possibly due to the fact that in these forms the sporangia mature and spores emerge on agar and thus fresh spores may be picked up and spread on fresh agar plates. In the greater number of chytrids tested the spores germinated and grew best on agars with small amounts of nutrients or even on plain agar.

In the present report only the forms which could be cultured are considered. Several other species were tested but failed to grow.

BOTANICAL LABORATORY,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

LITERATURE CITED

BUTLER, J. B., AND HUMPHRIES, A. 1932 On the Cultivation in Artificial Media of *Catenaria anguillula*, a Chytridiacean parasite, etc. Scientific Proc. Roy. Dublin Society **20** (n.s.): 301–324, pls. 13–18.

COKER, W. C. 1923 The Saprolegniaceae. Univ. of N. C. Press. Chapel Hill, N. C.

COUCH, J. N. 1931 Observations on Some New Species of Water Molds Connecting Achlya and Dictyuchus. Journ. E. M. Sci. Soc. **46**: 225.

HARVEY, J. V. 1925 A Study of the Water Molds and Pythiums Occurring in the Soils of Chapel Hill. Journ. E. M. Sci. Soc. **41**: 151–164, pls. 12–19.

KARLING, J. S. 1937 The Structure, Development, Identity and Relationship of Endochytrium. Amer. Journ. Bot. **24**: 352–364.

KARLING, J. S. 1938 A Further Study of Catenaria. Amer. Journ. Bot. **25**: 328–335, figs. 1–34.

SPARROW, F. K. 1931 Two New Chytridiaceous Fungi from Cold Spring Harbor. Amer. Journ. Bot. **18**: 615–623, pl. 45.

ZOPF, W. 1887 Über einige niedere Algenpilze (Phycomyceten) und eine neue methode ihre keime aus dem Wasser zu isolieren. Abhdl. Naturf. Ges. Halle **17**: 79–107.

JOURNAL

OF THE

Elisha Mitchell Scientific Society

Volume 55 December, 1939 No. 2

PROCEEDINGS OF THE THIRTY-EIGHTH MEETING OF THE NORTH CAROLINA ACADEMY OF SCIENCE

WAKE FOREST COLLEGE, WAKE FOREST, NORTH CAROLINA, MAY 5 AND 6, 1939

The thirty-eighth annual meeting of the North Carolina Academy of Science was held at Wake Forest College, Wake Forest, N. C., May 5 and 6, 1939.

At 9:30 a.m. on Friday, May 5, the General Section was called to order by the President John W. Lasley, Jr. The reading of papers was commenced and continued until 11:00 a.m. when the president appointed the following committees:

Auditing: G. Howard Satterfield, Paul J. Kramer, Albert F. Thiel.

Resolutions: Willard Berry, Z. P. Metcalf, J. N. Couch.

Nominating: H B. Arbuckle, W. E. Speas, H. R. Totten.

The reading of papers was then resumed and continued until 1:00 p m. when recess was taken for luncheon.

The afternoon meeting of the General Section commenced at 2:30 p.m. and continued until 4:30 p.m. when the Academy held its annual business meeting.

At the business meeting, the minutes of the last meeting were approved as published in the *Journal of the Elisha Mitchell Scientific Society* 54: 163–199. 1938.

Reports of the various committees were then called for as follows:

The executive committee, consisting of John W. Lasley, Jr., President of the Academy; Donald B. Anderson, Vice-President; H. L. Blomquist, Secretary-Treasurer; W. L. Porter, R. F. Poole, and O. C. Bradbury, reported as follows:

"The executive committee met in Durham on May 4 and again in Wake Forest on May 5 with all members present. The following matters were taken up by the committee:

"(1) Professor W. L. Porter was appointed to act as temporary chairman for the Botanical Section.

"(2) Four titles which arrived too late to be placed on the printed program were added to the program.

"(3) A petition by J. B. Derieux that his paper no. 26 be moved up and numbered '12B' was granted.

"(4) The committee reported as elected to membership since the last meeting the following:

Adams, Donald K., Dept. Psychology, Duke University.
Beasley, J. D., U. S. Dept. Agr., Cotton Lab., Raleigh, N. C.
Bolton, Robert L., Dept. Psychology, U. N. C.
Bridgeman, Ann J., Dept. Biology, Flora MacDonald College.
Britt, Henry G., Dept. Biology, Wake Forest College.
Bullock, R. C., Dept. Mathematics, N. C. State College.
Burton, Mary G., Dept. Botany, U. N. C.
Chase, Wilton P., Dept. Psychology, Woman's College, U. N. C.
Cocke, Elton C., Dept. Biology, Wake Forest College.
Coe, Joffre, Archaeology, U. N. C.
Cooper, Margaret, Dept. Chemistry, Meredith College.
Duffy, Elizabeth, Dept. Psychology, Woman's College, U. N. C.
Gauger, Herman C., Poultry Dept., N. C. State College
Gay, Roland A., Dept. Mathematics, Wake Forest College.
Gilbert, Gracie P., Campbell College.
Henderson, Edgar H., Dept. Philosophy and Psychology, Meredith College
Kramer, Margaret, Dept. Biology, Meredith College.
Kuhn, A. Burgin, Jr., Dept. Chemistry, Davidson College.
McKenzie, Robert M., Route #1, Gastonia, N. C.
Mackie, George C., (M.D.), Dept. Physiology, Wake Forest College of Medicine.
Moore, J. H., Dept. Plant Breeding, N. C. State College.
Morrow, Emmet B., Associate Horticulturist, N. C. State College.
Murchison, R. G., Dept. Geology, U. N. C.
Nature Study Club, Durham High School, President Jean Booth, sponsored by Dorothy Wilson.
Park, Hubert V., Dept. Mathematics, N. C. State College.
Physics-Chemistry Club, Durham High School, President John Blalock, sponsored by B. G. Stewart.
Pratt, J. G., Dept. Psychology, Duke University.
Rhine, J. B., Dept. Psychology, Duke University.
Shands, W. H., Oxford, N. C.
Slay, R. J., Dept. of Sciences, Eastern Carolina Teachers College.

Stump, Albert B., Dept. Biology, Flora MacDonald College.
Wladkowsky, Edith. Psychologist, Caswell Training School, Kinston, N. C.
Womack, A. W., Head Gardener, U. N. C.

"(5) The following former members were re-instated to membership:

Bruner, Stephen C., Estacion Experimental Agronomica, Santiago de las Vegas, Cuba.
Cameron, E. H., Dept. Mathematics, U. N. C.
Carroll, J. G., Dept. Mathematics, Wake Forest College.
Dashiell, J. F., Dept. Psychology, U. N. C.
Rayner, K. T., Dept. Mathematics, Wake Forest College.
Williams, Myra, Dept. Biology, Meredith College.
Zener, Karl, Dept. Psychology, Duke University.

"(6) The committee reported the following losses during the year:

Lost by death: H. V. Wilson.
Lost by resignation: Lula G. Winston.
Dropped from roll because of non-payment of dues: 12 former members.

The Treasurer's report was as follows:

Financial Statement of N. C. A. S., May 4, 1939

Receipts

Balance on hand May 5, 1938:		
Savings Account	$357.41	
Checking Account	118.42	
Cash on hand (dues)	8.00	
Total balance		$483.83
Dues:		
1938	212.00	
1939	245.00	
Initiation Fees:		
1938	64.00	
1939	52.00	
Chemistry Section contribution to program (1938)	5.00	
		578.00
Interest on savings		8.97
		$1,070.80

Disbursements

Stationery and printing	$57.83	
Programs (1939)	34.00	
Membership cards (1938)	4.20	
Membership cards (1939)	4.20	
Books for H. S. Essay Prize	18.08	
Express on books	.54	
Postage (stamps)	3.50	
Paper (bond)	3.45	
Contrib. to membership in Comm. Preserv. of Nat. Cond. Ecol. Soc.	5.00	
Charges on bank balance	.23	
Refund for overpaid dues	5.00	
Receipt books	1.03	
Flowers for Wilson funeral	5.15	
Secretarial assistance	58.00	
Refund of dues to Secretary	2.00	
Secretary's commission	53.50	
Jour. Elisha Mitchell Sci. Soc.	300.00	
Total disbursements		555.71
Total balance May 4, 1939		$515.09

Savings Account

Balance May 5, 1938	$357.41	
Interest	8.97	
Balance May 4, 1939		366.38
		148.71
Cash on hand		14.00
Balance in checking account		$134.71

The above report was made as of May 4, 1939.
Submitted by H. L. Blomquist, Secretary-Treasurer.
Audited May 5, 1938, by

G. HOWARD SATTERFIELD,
PAUL J. KRAMER,
ALBERT F. THIEL.

The committee accepted the invitation of Davidson College to hold the thirty-ninth annual meeting at Davidson, N. C., in 1940.

The executive committee then made the following recommendations to the Academy:

"1. That all bills presented in the Treasurer's report be authorized and paid and that the report be printed after being audited.

"2. That Bert Cunningham be appointed to select the books as the prize for the winner of the High School Science Essay Contest and that he be authorized to draw upon the treasurer an amount not to exceed $20.00 for these books. It was also recommended that the secretary be authorized to appoint a representative of the Academy to award the prize and to draw upon the treasury the payment of his expenses.

"3. That the secretary-treasurer be authorized to pay the Elisha Mitchell Scientific Society $300.00 each year for the publication of the Proceedings of the Academy so long as the finances of the Academy are in good condition.

"4. That the secretary-treasurer be authorized to order 50 reprints of the Proceedings for the purpose of exchange with other state academies.

"5. That the secretary-treasurer transfer an amount from the checking account to the savings account which will bring the latter up to $400.00. This amount was $33.62.

"6. That the following members be elected to life membership in the Academy:

H. B. Arbuckle, Davidson College.
W. C. Coker, The University of North Carolina.

Dr. Arbuckle joined the Academy in 1918, served as its president in 1920, and has been an active and valuable member.

Dr. Coker is a charter member of the Academy (1902) and served as its president in 1910. He has always been an active member and has served the Academy in the capacity of Chairman of the Editorial Board of the *Journal of the Elisha Mitchell Scientific Society*, the official organ of the Academy.

The recommendations of the executive committee were approved.

The auditing committee reported that the Treasurer's report had been examined and found to be correct.

The reports of the auditing committee and the Treasurer's report were accepted and approved.

The report of the high school science committee was as follows:

This committee has undertaken to stimulate a greater interest among high school teachers and students during the past year in at least two directions.

As in former years, it has sponsored the essay contest. Through con-

tact with the teachers at each of the district meetings, and through form letters the matter of the essay contest was presented directly to the teachers. The response was reasonably good, some 40 or 50 teachers indicating their desire to have their students compete. The final results, that is the essays submitted, were thoroughly disappointing in number and quality. The report of the judges will be given presently.

During the year your committee has formulated plans by which the essay contest will be replaced by a project contest. The provisional detailed plans are available to any interested members of the Academy.

With the permission of the Academy, the committee has arranged a program for high school science teachers, which will be in session at Wake Forest on Saturday morning. The response has been good, but the value of this meeting can not be estimated until after tomorrow's session.

The committee suggests a more cooperative spirit on the part of members of the Academy with the high school program. In times past this committee has requested the cooperation of college teachers in giving lectures to high school groups. Less than half a dozen have responded. The chairman of this committee has received several requests for lectures, and has referred the inquirer to certain persons who might be available, always in doubt of course, as to whether or not the person is available. A list of lecturers and subjects would be especially valuable if this project is to be at all useful. This is especially true in light of the rapid development of the high school science clubs in the state. The committee requests that any persons willing to perform this service will communicate with the chairman.

Recent visits with high school teachers indicate that sets of lantern slides accompanied by a "script" could be very helpful to high school teachers. The committee hopes that members of the Academy who can provide such service will communicate with the chairman of the committee.

Your committee has also made a preliminary survey of the science clubs in the state and has learned of some thirty that are now functioning. This number will probably double during next year and the Academy should be looking forward to their confederation in order that the highest good may come from them. A few of these have already affiliated with the Academy under the provision adopted at our last meeting. Others may be expected to affiliate in the fall. In the light of our present knowledge it appears as if the Academy will be called upon within the next year or two to consider the advisability of establishing

a Junior Academy to be made up of the members of these high school clubs.

In accordance with the criteria set up the committee nominated Ralph Kiser, Central High School, Charlotte, N. C., Miss Eugenia Cox, Old Town High School, Winston-Salem, N. C., for Junior Membership in the A. A. A. S. They were duly elected by the A. A. A. S.

The committee recommends the authorization of a budget for the project contest similar to that of the past for the essay contest.

The committee recommends that the Secretary of the Academy select a member of the Academy to deliver this year's prize and that the expense thereof be borne by the Academy.

The committee recommends that the Secretary of the Academy appoint a member of the Academy to select the books for this year's award.

Respectfully submitted,
BERT CUNNINGHAM,
C. E. PRESTON,
R. J. CAMPBELL,
MARY CONRAD CLEAVER,
J. H. HIGHSMITH,
R. J. SLAY,
H. B. ARBUCKLE.

The judges of the high school essays reported as follows:

"The number of essays submitted this year was disappointingly small and the quality of most of them was lower than in past years.

One essay, however, was outstanding and of distinctly good quality. It is recommended that the prize be awarded to Mr. Alfred Gallant of Charlotte Central High School, Charlotte, North Carolina, for his essay "Quartz Crystals for Radio Transmitters."

PAUL J. KRAMER,
J. L. STUCKEY,
D. P. COSTELLO, acting
for C. D. BEERS,
Judges

The report of the high school science committee and that of the judges of the essays were accepted.

The legislative and elective appraisal committees had no reports to make at this meeting.

The conservation committee reported as follows:

Your committee has continued its efforts to secure the establishment of permanent natural areas in North Carolina. In compliance with the resolution endorsed last year by this Academy the Chief Forester of the United States Forest Service was requested to do everything possible to complete the dedication of the Black Mountain Natural Area, which request has now been complied with. It seems that the original tract recommended for this natural area was larger than the authorities were willing to approve. The area, therefore, was reduced to some 1405 acres and was officially approved last summer. In a letter from the Regional Forester he states that the Black Mountain natural area as officially approved includes only the watershed of Middle Creek: "The correct description of the boundary of this area is as follows: The watershed is bounded on the west by the summit of the Black Mountains between Balsam Cone and Potato Hill, on the north by Middle Ridge, on the south by the north branch of Maple Camp Ridge, and on the east by the National Forest boundaries." The Regional Forester further says: "In case you visit this area, it is suggested that you use State Highway NC #104 and take the trail up Maple Camp Ridge to the top of the mountains, then follow the top of the mountains to Potato Hill and turn east down Middle Ridge by the trail which comes down the valley to Colbert Creek." A map was furnished showing its location but the request for photographs has so far had no response. It is probable that there are no photographs which can be definitely said to have been taken on this area. The primary object of this natural area, the elevation of which ranges from 3500 to 6600 feet, was to retain a specimen of the original forest of red spruce and southern balsam which formerly covered most of the higher elevations in the Southern Appalachians. An ecological study of this area for this Academy would prove most interesting. I might add that the spruce forest on this natural area can be most easily reached by driving to the parking place on Mount Mitchell State Park and taking the trail north along the Black Mountain divide to Balsam Cone, a distance of some two miles.

The request for the acquisition by the United States Forest Service of the Ravenel virgin forest at Highlands for inclusion in the Nantahala National Forest has been less successful. The reduction of the congressional appropriations for land purchase is no doubt largely responsible. Interest in the saving of an adjoining tract of 289 acres has recently resulted in an appeal to the Chief of the Forest Service by Dr.

W. C. Coker, followed by a request to certain members of Congress by this committee for aid in securing the acquisition of this property known as the Richardson Woods which has usually been considered a part of the well known Primeval Forest. The purchase of these woods by a lumber company has been arranged and only prompt action can save it from devastation. Dr. Coker describes this area as follows: "This forest averages about 4,000 feet elevation and consists of two holdings, one the "Richardson Woods" (289 acres), the other the "Primeval Forest" (1600 acres). These two areas are contiguous and together make up the finest example of untouched forest growth in the eastern United States. Unless you have seen this forest you cannot grasp its grandeur. I have seen nothing like it outside of the Pacific Slope. In these forests there are to be found the largest specimens of several species of eastern American trees, such as Carolina and Canadian Hemlock, Black Cherry, Silverbell, (*Halesia*). Here also the Cherry Birch (*Betula lenta*), Fraser's Magnolia, and Cucumber Tree probably reach their greatest development Adjacent to these forests for some distance is the Nantahala National Forest." "It is estimated that the timber on this tract includes 4,500,000 feet of hemlock; 130,000 of poplar; 300,000 of oak; 255,000 maple and birch; 2500 of cherry, and 1,000,000 of chestnut " It is hoped that this wonderful tract of virgin timber may yet be saved Members of the Academy can help by writing the Chief Forester or the National Forest Reservation Commission, or their individual congressmen.

The Ravenel Forest of 1600 acres adjoining the Richardson Woods and within the Nantahala National Forest should also be acquired and given natural area status. It is at present safe from the lumberman's axe but continued interest in preserving it is very important.

In the report of this committee a year ago the summit of Roan Mountain was recommended as a desirable site for a publicly-owned natural area. I am glad to report that definite steps seem to have been taken to include this very interesting area in the Pisgah National Forest.

A new line of endeavor for this Academy has been suggested through some correspondence with the Virginia Academy of Science which was referred to this committee by Dr. Bert Cunningham of Duke University. Dr. E. C. L. Miller, Secretary of the Virginia Academy, wrote Dr. Cunningham April 20 asking information about the condition and ownership of that portion of the Dismal Swamp lying in North Carolina. He says: "There is some interest here in Virginia just now concerning the future of the Dismal Swamp. The Izaak Walton League of Virginia

has already taken action requesting its National Executive Committee to propose to the Department of the Interior the acquisition of the Dismal Swamp Area in southeastern Virginia. They have also requested the Virginia Academy of Science to support the movement, and it will probably do so at our Danville meeting in May. I wonder if you could stir up some interest in North Carolina?" Dr. Miller's suggestion is that the North Carolina Academy of Science and the Virginia Academy could cooperate in planning some worthwhile future for the Swamp.

"Planning some worthwhile *future*" is very necessary and the best thing that can be done. The *present* condition of the Dismal Swamp is lamentable. Most of the better timber has been taken out; logging has in most areas been followed by fire which has destroyed the seedlings which would have started re-establishment of the forest cover; the white cedar, which has been the most valuable tree, has been succeeded by what are known as "lights," namely areas covered with shrubs and reeds interlocked with cat-briar and other vines. In most cases these shrubs are so dense that the more valuable tree species cannot become re-established. A preliminary study was made some eighteen years ago by two members of this conservation committee, namely Dr. C. F. Korstian and the present chairman. A follow-up study should bring out much valuable information. It is suggested by your committee that it devote as much time as possible to a study of the natural and economic conditions of the Dismal Swamp and report progress to the next meeting of this Academy. It is also suggested that this committee be authorized to cooperate with the Virginia Academy of Science in this study insofar as our common interests may be promoted.

In closing the committee desires to offer the following resolutions:

Resolution I. Richardson Woods.

WHEREAS, the Richardson Tract of 289 acres and the Ravenel Tract of 1600 acres, known together as the Primeval Forest has exceptional and unique qualities which should be preserved for the study, information and pleasure of future generations, and

WHEREAS, the Richardson Tract is now in imminent danger of being cut over for lumber and its interest destroyed, and

WHEREAS, this area is adjacent to land already acquired by the Federal Government as part of the Nantahala National Forest and itself lies within the purchase area; therefore, be it

Resolved that the North Carolina Academy of Science does hereby urge upon the Forest Service of the United States Department of Agriculture and upon the National Forest Reservation Commission the

importance of acquiring this tract immediately and adding it to the Nantahala National Forest.

Resolved further, that a copy of this Resolution be submitted to the President of the United States, to the Secretary of Agriculture, to the members of the National Forest Reservation Commission, and to the North Carolina delegation in Congress.

Resolution II. Cooperation with Virginia Academy of Science.

Resolved, that the North Carolina Academy of Science herewith expresses its appreciation of the invitation of the Virginia Academy of Science implied in the letter of its Secretary, of April 20, 1939, and herewith authorizes the Conservation Committee to arrange and carry out such cooperation as may be of mutual benefit to the two Academies in the proposed study of the Dismal Swamp Area.

Respectfully submitted,
J. S. HOLMES,
W. C. COKER,
J. P. GIVLER,
C. F. KORSTIAN,
H. J. OOSTING,
Committee.

The report of the conservation committee was accepted and the resolutions recommended therein adopted.

As representative of the Academy Conference, Bert Cunningham, made the following report:

"Several matters of considerable importance were discussed at the meeting.

"1. Time allotted for the meeting. This was felt to be insufficient and measures were adopted for an increase.

"2. Temporary and last minute appointment of representatives. The work of the Conference depends largely upon a continuing membership. Academies were requested to appoint representatives for not less than a three-year period. They were also urged to send an alternate if the regular appointee could not attend. It seemed better to elect the delegate than to appoint, since in the latter case the appointment was valid only for the term of the Academy president.

"3. A paper on the "Status" of Academies shows a great lack of cooperation on the part of several Academies. Representatives were urged to bring this matter to the attention of the various Academies.

"4. The need for data for intelligent discussion. This calls for

preparation of papers well in advance of the meeting. Plans were set on foot at the meeting for next year's program.

"5. The problem of Junior Academies. This was forcefully brought into the picture by the activities of the American Institute of Engineering and Science. A committee (of which your representative was a member) was set up to discuss the matter with representatives of the Institute. This committee held an all day session in Indianapolis and arrived at a satisfactory solution of the problem. A detailed report of the committee's work will later be filed with the Secretary of the Academy.

"6. Co-operation with the American Association. It was pointed out that thus far most of the co-operation had been on the part of the Association. Academies were urged to make an effort to bring more of their members into the Association. This would in return bring larger grants for research to the Academy. It was indicated by the Association that it would prefer to make its research grants through the Academies, if they could show that they were capable of handling them.

"7. Research grants. A "one man" committee was set up to make an annual report of these grants.

"8. Junior Memberships. A committee was set up for annual reports on this project. Considerable interest was manifested as to the manner in which the nominees were selected. This will be a subject for report at the next meeting.

"9. Miscellaneous: Several other matters presented in a paper by your representative (a few copies of which are available for Academy members) were discussed briefly, and plans were made for further discussion.

"10. Your representative was elected Conference President for the ensuing year.

Respectfully submitted,
BERT CUNNINGHAM.

The report of the representative to the Academy Conference was accepted.

The committee on the A. A. A. S. Research Grant reported that the research grant for 1939 had been awarded to F. H. McCutcheon, Department of Zoology, N. C. State College, for use in his "Investigation of possible experimentally induced seasonal variations in the oxygen affinity of hemoglobin in mammals." The report was adopted.

As the representative of the Academy on the Council of the American

Association for the Advancement of Science, Bert Cunningham made the following remarks:

"Since the reports of the Council have been published, it seems unnecessary for your representative to make any report other than that he attended all meetings of the Council."

The committee on the Academy Medal reported that the medal for 1939 had been awarded to F. H. McCutcheon for his paper entitled "The respiratory mechanism of the grasshopper."

The following memorial report honoring the memory of Henry Van Peters Wilson was presented:

HENRY VAN PETERS WILSON

In the death of Professor H. V. Wilson on January 4, 1939, the North Carolina Academy of Science lost one of its founders and one of its most distinguished and valued members. Henceforth his personal charm and his stimulating presence will be severely missed at our annual meetings.

Henry Van Peters Wilson was born in Baltimore, Maryland, on February 16, 1863, and was a son of Reverend Samuel A. Wilson and Sophia Anne Stansbury Wilson. He received his formal education in the schools of Baltimore, at the Baltimore City College, and at Johns Hopkins University. In 1883 he was awarded the degree of Bachelor of Arts by Johns Hopkins University and in 1888 the degree of Doctor of Philosophy. From 1889 to 1891 he was an investigator for the United States Fish Commission at the Woods Hole laboratory. From 1891 until his death he was successively Professor of Biology, Professor of Zoology, and Kenan Professor of Zoology in the University of North Carolina.

Dr. Wilson's was a forceful personality, and he was widely recognized as a man of superior qualities of character and mind. He was a cultured gentleman with an enthusiasm for scholarship and intellectual exploration, and he believed that rigorous thinking had a high value in civilizing the individual. Accordingly he encouraged intellectual habits in others, and as a teacher insisted upon precision in observation and logical thinking in the analysis of data obtained from observation. As a teacher he possessed fine capacity and a willingness to take no end of pains in directing and criticising the work of those students whom he considered worthy to enter upon a scientific career. The young men and women who worked under him received not only meticulous training but scientific spirit and breadth of vision. It was part of his genius as a teacher to be inspiring without effort and to convey to his students

the realization that science has much to contribute to human culture and is worthy of a man's best efforts.

He was far from being a mere dilettante playing at science and drawing gratuitous pleasure from the discoveries of others; on the contrary, he embodied the attitude of the spirited professional worker who looks upon research as a thrilling adventure, yet one that must be carried out methodically and with foresight. There resulted from his researches a long list of contributions to biology dealing chiefly with the taxonomy and adaptations of sponges and with embryology and regeneration. (a list of his publications from 1891 to 1933 may be found in the Journal of the Elisha Mitchell Scientific Society, volume 50, and a supplemental list in volume 55.)

At every opportunity Dr. Wilson was active in promoting the research spirit and research facilities. In addition to his participation in the activities of the national academies and societies, he was an active and helpful member of the Elisha Mitchell Scientific Society, the University of North Carolina chapter of Sigma Xi, and the North Carolina Academy of Science. He was instrumental in the founding of the latter two of these organizations and served as president of each of them. Steadily, as the resources of the University permitted, he built up the library, laboratory, and staff of his department until its value was recognized nationally. Having worked at the old Johns Hopkins laboratory at Beaufort when he was a student, he was aware of the resources and attractions of that location for a seashore laboratory. It was largely through his efforts that there was established at Beaufort the United States Bureau of Fisheries Biological Station, an institution in which he always maintained a great interest. This laboratory has been of great value in the development of biological science in America as is attested by the long series of important researches that have emanated from it. Furthermore, it has provided opportunity and inspiration to many young investigators during their formative years.

In his hours of relaxation Dr. Wilson was a delightful and stimulating companion. He was not only a zoologist but a philosopher and an alert human being with a keen interest in literature and art and the activities of other human beings. His conversation moved pleasantly from topic to topic and he was always entertaining. In the words of one of his contemporaries and lifelong friends, "His was a rare spirit. The loss of his friendship is a saddening experience."

In 1893 he married Edith Theresa Stickney, of Boston. From this

marriage there were three children, Edith Stedman (Mrs. Thorndike Saville), Eleanora Stansbury (Mrs. Howell Peacock), and Dr. Henry Van Peters Wilson, Jr., all of whom survive him. To them and to his sister the North Carolina Academy of Science extends its deepest sympathy.

C. S. BRIMLEY,
J. P. GIVLER,
A. S. PEARSE,
W. L. PORTER,
W. C. GEORGE (*Chairman*),
Committee from North Carolina Academy of Science

C. E. Preston then made an announcement concerning the science instruction of the National Education Association.

The appointment of the following committees were then made by the president:

Legislative Committee: H. F. Prytherch, chairman, B. W. Wells, W. F. Prouty.

Conservation Committee: J. S. Holmes, chairman, J. P. Givler, C. F. Korstian, W. C. Coker, H. J. Oosting.

High School Science Committee: Bert Cunningham, chairman, J. H. Highsmith, B. B. Brandt, Mary Conrad Cleaver, R. J. Slay, John W. Wood, C. F. Dodson.

Committee on the Academy Medal Award: O. C. Bradbury, chairman, E. W. Berry, Edward Mack, Jr., P. J. Kramer, J. P. Givler, J. B. Derieux, O. J. Thies, Jr., and Bert Cunningham (*ex officio*).

The Elective Appraisal Committee: P. M. Ginnings, chairman, E. H. Hall, R. E. Coker, Mary Conrad Cleaver, C. W. Edwards, Karl H. Fussler, E. G. Purdom, and R. N. Wilson.

The nominating committee submitted the following nominations:

President: H. L. Blomquist, Duke University.

Vice-President: John N. Couch, The University of North Carolina.

Secretary-Treasurer (three years): Bert Cunningham, Duke University.

New member on the Executive Committee (three years): Earl H. Hall, Woman's College, The University of North Carolina.

One member on the Committee of the A. A. A. S. Research Grant (three years): B. W. Wells, North Carolina State College.

Representative to the Council of A. A. A. S.: Bert Cunningham.

Representative to the Academy Conference: Bert Cunningham.

The nominations were accepted and the secretary was instructed to cast the ballots for the nominees.

The general resolutions committee made the following report:

"The North Carolina Academy of Science wishes to express its appreciation for the delightful entertainment received at Wake Forest College. We desire to express our appreciation to the local committee on arrangements, to Wake Forest College for its complimentary dinner, to Miss Eleanor Mayes who has so generously assisted with the registration, and the students of Wake Forest College who have assisted in routine matters."

WILLARD BERRY,
Z. P. METCALF,
J. N. COUCH,
Committee.

The business meeting then adjourned.

At 7:00 p.m. the Academy was entertained at a complimentary supper.

At 8:30 p.m. the evening meeting was held in the auditorium of the Medical Building with Donald B. Anderson, Vice-President, presiding. In the absence of T. D. Kitchin, President of Wake Forest College, the address of welcome was made by Dean B. D. Bryan. This was followed by the presidential address, "The relation between mathematics and science," by the retiring president, John W. Lasley, Jr.

On Saturday morning the Academy met in the following sections: General, Botany, Mathematics, Physics, Zoology, The North Carolina Section of the American Chemical Society, and the High School Science Teachers.

The following officers were elected by the various sections:

North Carolina Section of the American Chemical Society:

Chairman: E. C. Markham, The University of North Carolina.

Vice-Chairman: W. E. Jordan, N. C. State College.

Secretary-Treasurer: Ivan D. Jones, N. C. State College.

Mathematics Section: *Chairman:* T. F. Hickerson, The University of North Carolina. *Secretary:* J. A. Greenwood, Duke University.

Physics Section: *Chairman:* H. E. Fulcher, Davidson College. *Secretary:* F. W. Lancaster, N. C. State College.

Zoology Section: *Chairman:* J. P. Givler, Woman's College, U. N. C. *Secretary:* Z. P. Metcalf, N. C. State College.

High School Science Teachers Section: *Chairman:* Clifford Beck, Salisbury, N. C. *Secretary:* Harry MacDonald, New Bern, N. C.

The following papers were presented. Those marked * appear in full in this issue; those marked x are abstracted with the Proceedings; those marked † were read by title.

GENERAL SECTION

x1. *Atmospheric pollen survey of Danville, Va.* (Lantern). ELTON C. COCKE, Wake Forest.

2. *Investigation of the Fluoride Content of North Carolina School Water Supplies* (Lantern). E. E. RANDOLPH, N. C. State.

3. *Weather conditions on Mount Mitchell, N. C.* LEE A. DENSON, U. S. Weather Bureau, Raleigh.

4. *Diurnal temperature variations within full-sized cotton bolls* (Lantern). DONALD B. ANDERSON, N. C. State.

5. *Varietal differences in the vitamin C content of cantaloupes* (Lantern). M. A. MOSELY AND G. HOWARD SATTERFIELD, N. C. State.

6. *Hardening peanut-fed pigs* (blackboard). E. H. HOSTETLER, J. O. HALVERSON AND F. W. SHERWOOD, N. C. State.

7. *Pressures in bottled carbonate liquids at low temperatures* (Lantern). O. J. THIES, JR., AND H. E. FULCHER, Davidson.

8. *The Japanese beetle suppression program in North Carolina* (Lantern). C. H. BRANNON, U. S. Dept. Agr., Raleigh.

x9. *The effect of manganese and copper on tobacco* (Lantern). W. B. RANKIN AND L. F. WILLIAMS, M. C. State.

x10. *Respiratory mechanism in the grasshopper* (Lantern). F. H. McCUTCHEON, N. C. State.

11. *The present status of the magnetic lens electron microscope* (Lantern). OTTO STUHLMAN, JR., U. N. C.

x12. *Miles per dollar with different grades of gasoline* (Lantern). J. B. DERIEUX, N. C. State.

x12b. *Efficiency of an aspirator type of basement water drainer* (Lantern). J. B. DERIEUX, N. C. State.

x13. *Development and distribution of tobacco roots in relation to soil profile* (Lantern). L. J. GIER, Campbell College.

14. *Physiological responses to physical factors in environment* (Lantern). F. G. HALL, Duke.

x15. *Sex chromosomes in plants; a cytological hoax* (Lantern). H. W. JENSEN, Asheville Farm School.

16. *New measurements of the rate of loss of heat by the earth to space* (Lantern). CHARLES M. HECK, N. C. State.
17. *Some new species of Basidiomycetes.* W. C. COKER, U. N. C.
x18. *Remarks concerning a natural history survey of the Great Smoky Mountains National Park* (Lantern). ARTHUR STUPKA, National Park Service.
*19. *Lochetic luminous dipterous larvae* (Lantern). B. B. FULTON, N. C. State.
x20. *Distribution of the fiber population on the cotton seed* (Lantern). JERRY H. MOORE, N. C. State.
†x21. *Archaeology in North Carolina.* JOFFRE COE, U. N. C.
†22. *Geology of the northern half of Wake County.* J. L. STUCKEY, N. C. State.
†x23. *Mechanical analysis of Triassic sediment* (Lantern). WILLARD BERRY, Duke.
x24. *Vitamin A in sheep's milk and colostrum* (Lantern). R. E. CLEGG AND G. HOWARD SATTERFIELD, N. C. State.
25. *Vitamin C in fresh and processed cucumbers.* M. F. SHOWALTER AND G. H. SATTERFIELD, N. C. State.
†26. *The relationship between vocational factors and values factors in college women.* ROBERT L. BOLTON AND W. J. E. CRISSY, U. N. C.
†27. *Size of stimulus symbols in extra-sensory perception.* J. G. PRATT, Duke.

BOTANY SECTION

x1. *Studies of the fruiting habit of the peanut.* G. K. MIDDLETON AND PAUL H. HARVEY, N. C. State.
x2. *The production of new species of cotton* (Lantern). J. O. BEASLEY, U. S. Dept. Agr. Cotton Lab., Raleigh.
3. *A mosaic disease of cowpea* (Lantern). S. G. LEHMAN, N. C. State.
x4. *The manner in which excess of CO_2 reduces water intake in plants* (Lantern). P. J. KRAMER, Duke.
5. *Heterothallism in the Chytridiales* (Lantern). JOHN N. COUCH, U. N. C.
x6. *Cytology of a new species of the Plasmodiophoraceae.* ALMA J. WHIFFEN, U. N. C.
x7. *Vegetative regeneration in the West Coast Manzanitas* (Lantern). J. E. ADAMS, U. N. C.

8. *The relationship of Pseudolpidium and Olpidiopsis* (Lantern). LELAND SHANOR, U. N. C.
x9. *Peat formation in the Carolina Bays* (Lantern). MURRAY F. BUELL, N. C. State.
10. *Clavochytridium;* a new genus of the Chytrids. HIDEN T. COX, U. N. C.

ZOOLOGY SECTION

x1. *A new trematode from Chelydra serpentina* (Lantern). H. G. BRITT, Wake Forest.
2. *Sex determination in the parasitic wasp, Habrobracon juglandis* (Lantern). C. H. BOSTIAN, N. C. State.
3. *A new species of Brachylacmus (Trematoda) from the barred owl* (Lantern). REINARD HARKEMA, N. C. State.
4. *An effective Heidenhain's hematoxylin staining technique for certain intestinal protozoa, particularly Endamoeba coli cysts.* LAWRENCE S. RITCHIE, W. C. of U. N. C.
5. *Degenerative and regenerative changes in the rat testis following infra-red treatment* (Lantern). W. L. WILLIAMS AND BERT CUNNINGHAM, Duke.
*6. *Metabolism and sex in the hen's egg* (Lantern). BERT CUNNINGHAM, Duke.
x7. *The openings of the seminal receptacles of earthworm* (Lantern). E. C. COCKE, Wake Forest.
8. *What is in a name?* (Lantern). Z. P. METCALF, N. C. State.

MATHEMATICS SECTION

x1. *Note on a sampling restriction.* J. A. GREENWOOD, Duke.
x2. *On loci associated with osculants and penosculants of a plane curve.* E. A. CAMERON, U. N. C.
x3. *Conditions on two singular matrices A and B, such that AB and BA may have the same reduced characteristic function.* H. V. PARK, N. C. State.
x4. *Conditions that a matrix B be expressible as a polynomial in a set of partial idempotent and nilpotent elements of a given matrix A.* H. M. NAHIKIAN, N. C. State.
5. *Solution of multiple simultaneous linear equations.* T. F. HICKERSON, U. N. C.

PHYSICS SECTION

x1. *The relation between the reading and the area of exposure of a Weston Photronic Foot Candle Meter* (Lantern). J. B. DERIEUX, N. C. State.

x2. *The production of a uniform magnetic field in a long rectangular box.* ARTHUR E. RUARK, U. N. C.

x3. *On the variations in wave form at different points in the tank circuit of a H.F. vacuum tube oscillator* (Lantern). SHERWOOD GITHENS, JR., Wake Forest.

4. *Phenomenon of a series resonance circuit.* FORREST W. LANCASTER, N. C. State.
5. *Some observations on the effect of surface oxide films on electrode potentials of aluminum and chromium.* W. E. SPEAS, Wake Forest.
6. *Penetration in the atmosphere of the hard component of the cosmic radiation.* L. W. NORDHEIM, Duke.
7. *Geiger-Mueller counter demonstration of the penetrating power of alpha, beta, gamma rays.* C. M. RYERSON, Duke.

NORTH CAROLINA SECTION OF THE AMERICAN CHEMICAL SOCIETY

1. *Identification of complex ions in solution by means of the spectrophotometer.* W. C. VOSBURGH AND GERALD R. COOPER, Duke.
2. *Aqueous solubilities of some unsaturated alcohols.* P. M. GINNINGS, ELEANOR HERRING AND DORIS COLTRANE, Greensboro College.
3. *The formation and composition of Pinene gum.* BERNARD BERGER, U. N. C.
4. *Vitamins A and B in soybeans and cowpeas.* F. W. SHERWOOD AND J. O. HALVERSON, N. C. State.
5. *Some derivatives of alkyl sulfonic acids.* P. H. LATIMER, U. N. C.
6. *The lipids of certain pathogenic fungi.* R. L. PECK AND C. R. HAUSER, Duke.
7. *The dielectric constant of mixtures of some polar liquids.* ELMER F. DRAKE AND MARCUS E. HOBBS, Duke.
8. *The vitamin C content of ten varieties of watermelons* (Lantern). G. HOWARD SATTERFIELD AND R. E. CLEGG, N. C. State.
9. *Some changes in brine composition occurring during the salting of cucumbers.* IVAN D. JONES, N. C. State.

HIGH SCHOOL SCIENCE TEACHERS

†1. *The four-year integrated science course.* E. R. ROBINSON, Greenville.

2. *Science clubs in North Carolina.* CAROLINE POWELL, Salisbury.
3. *Making the public more science conscious.* JOHN W. WOOD, Old Town.
4. *A high-school science library and its use.* HARRY MACDONALD, New Bern.
5. *A community museum.* GEORGE W. ROSS, Washington.

EXHIBITS

Special Exhibits

An effective Heidenhain's hematoxylin staining technique for certain intestinal protozoa, particularly Endamoeba coli cysts. LAWRENCE S. RITCHIE, W. C. of U. N. C.

The following abstracts have been received.

Atmospheric Pollen Survey of Danville, Virginia. ELTON C. COCKE.

An accurately determined pollen survey furnishes the best clue to the hay fever potentialities of any given locality. The pollen cycle is repeated year after year with only slight variations due to seasonal conditions. The pollen content of the atmosphere at Danville, Virginia, for 1936-37 is discussed. The data for this study were obtained from the results of daily count of the pollen grains caught on slides coated with glycerine jelly and exposed to the air for 24-hour periods. Arboreal forms shed their pollen from February 1 through May, reaching a maximum about May 1. During June and July grasses, plantains, and docks were the principal forms of vegetation shedding sufficient pollen to be recorded on pollen plates. On August 2 ragweed began to appear on the pollen slides. The concentration of this pollen increased rapidly until on the 12th of September, when it reached a maximum of 5000 per cubic yard. The pollen content of the air diminished rapidly from this date to the end of September. During October scattered grains of ragweed, grasses, chenopods, goldenrods, and other weeds were caught, but never in appreciable quantities.

Assuming twenty cubic yards as the normal intake of air per person per day, then during the maximum period of tree pollination persons in Danville inhaled from 50,000 to 500,000 pollen grains daily. The average number of ragweed pollen breathed in per day during the ragweed pollinating season was about 30,000 grains; on the day of maximum production of ragweed pollen approximately 100,000 granules entered the respiratory tract of each person in this area. The pollen production at Danville is not unusually high, in fact it is probably lower than many areas of the United States.

The Effect of Manganese and Copper on Tobacco. W. B. RANKIN AND L. F. WILLIAMS.

Tobacco plants growing in nutrient solutions were treated with varying concentrations of copper and manganese. The influence of the salts was measured by growth response, curing properties of the leaves, dry weight determinations, and analyses for copper, manganese, reducing sugars, and chlorophyll.

Seeds from plants which had previously shown much cherry leaf were sown in soil, and the per cent of cherry leaf which developed upon curing was determined. This preliminary investigation led to the conclusion that cherry leaf may be an inherited phenomenon. Copper and manganese absorption from soil was studied.

In the proper concentration manganese stimulates reproductive growth and increases dry weight; in the concentrations studied copper retards reproductive growth and decreases dry weight. The application of copper sulfate to all soils as a component of the general fertilizer is a highly questionable procedure.

It was not possible to demonstrate any correlation between the presence or absence of copper or manganese and the occurrence of cherry leaf.

The Respiratory Mechanism in the Grasshopper. F. H. MCCUTCHEON.

The present explanation of respiration in the grasshopper states that the respiratory cycle consists of two parts: (a) an inspiratory phase during which the anterior four spiracles are open and the last six closed, and (b) an expiratory phase during which the opening of the spiracles is reversed. Such a mechanism does not provide means for supplying air rapidly to more remote parts of the tracheal system. Here the progressively smaller tubules cause an increasingly higher resistance to the passage of air.

A study of the correlation between abdominal movements and the opening and closing of the spiracular valves was made with the aid of kymograph records. It showed the mechanism to be more complex than the one described above. Normal and dyspneic breathing were found to differ with reference to the nature of abdominal movements, but three distinct phases were always distinguishable in the respiratory cycle. These phases are (a) inspiratory, (b) compressatory, and (c) expiratory. The second phase, to which the name *compressatory* is given, appears to provide the necessary mechanism to force air to the smaller tracheal branches.

A capillary manometer sealed to the second spiracular opening demonstrated the efficacy of the compressatory phase in creating pressure in the tracheal system.

So far as physiological specialization for greater activity is concerned, the compressatory phase might be considered the most important part of the respiratory cycle in insects.

Miles per Dollar with Different Grades of Gasoline. J. B. DERIEUX.

The three standard grades of gasoline, "Premium," "Regular," and "White," were tested. In the method used the fuel pump was disconnected from the regular pipe leading to the automobile tank, and connected to a rubber tube leading into a 500-cubic centimeter graduated cylinder filled with the gasoline under test. Readings of the amount consumed were taken for a travel of a measured mile forward and return, which procedure practically eliminated grade and wind effect. Readings were taken at different speeds from five to sixty miles per hour.

The mileage of each rose rapidly as the speed increased until the speed reached about twenty-five miles per hour, after which it gradually fell off. At the highest mileage value the "Premium" gave about eighty-one miles per dollar, the "Regular," ninety-three, and the "White," one hundred and seventeen At ten miles per hour the respective values were seventy-five, eighty-five, and one hundred and two. At fifty miles per hour, they were sixty-five, seventy, and ninety.

Efficiency of an Aspirator Type of Basement Water Drainer. J. B. DERIEUX.

The drainer was connected at the proper end by a length of rubber hose to the city water supply and to the exhaust end of it was connected a 15-foot length of $\frac{3}{4}$-inch hose. The drainer was then laid in the bottom of a wash tub filled with water, which had a depth of one foot. Measurements of water exhausted and water consumed were made with the delivery end of the exhaust hose at elevation intervals of one foot, which was the top of the tub, to twelve feet, the latter being the maximum elevation at which exhaust would take place.

The volume exhausted in each case was determined by measuring the amount to refill the tub after each run. The volume consumed was determined by measuring the gross amount from the exhaust, and subtracting from it the amount exhausted from the tub. Each run was of one minute duration.

As to the *amount* of each water, graphs were plotted with elevation

and volume as axes. The graph of the *exhausted* sloped downward with increasing elevation, and was almost straight, but curved slightly with the concave side upward. This exhaust graph, extended to zero elevation, gave 13 quarts per minute at zero elevation, and as mentioned, before, zero quarts at twelve feet of elevation. The *volume consumed*, was practically constant at all elevations, which was 8½ quarts per minute, the graph of which, of course, was a straight line parallel with the elevation axis.

The efficiency was considered as the ratio of the exhaust volume to the net consumed volume. The graph, with height of exhaust and percentage as axes, was similar to the graph of the amount, at zero height the percentage was 150 and at twelve feet it was zero. The 100 per cent value came at an elevation of about four feet.

Distribution and Development of Tobacco Roots. L. J. GIER.

A number of plants were removed from the beds by the soil block washing method and from the field by the trench method. Drawings and measurements were made of the root systems and the plants dried for shoot-root ratio studies. The ratio was found to be near 10:1 through the entire season with a consistently high correlation between the dry weights of shoots and roots. Mature plants were found to have about 1400 feet of roots. Soil type seemed to have little effect on the root pattern, but depth of plowing did change the distribution of feeders.

Sex Chromosomes in Plants: A Cytological Hoax. HENRY WILHELM JENSEN.

Two paramount considerations upon which the theory of sex chromosomes has been built are discussed. From investigations upon the pre-meiotic chromosomes of *Rumex acetosella, Rheum Rhaponticum,* etc., doubt is cast upon the continuous individuality of the chromosomes in these species. The meiotic chromosomes appear to originate from a fundamentally continuous spireme which splits into two strands, out of which the haploid number of chromosomes later become segmented. Under such conditions sex chromosomes cannot be conceived.

A review of the literature on sex chromosomes in the Angiosperms shows that these peculiar meiotic phenomena are generally found in plants belonging to large and variable genera in which both hermaphroditic and unisexual species abound. The origin of these unisexual species appears to be relatively recent and through the agency of

hybridization. Investigation of meiosis in *Rumex acetosella, R. acetosa, Lychnis alba, Smilax rotundifolia, Dioscorea quarternata,* and *Ilex opaca* give ample evidence of previous hybridization of the species. The absence of sex chromosomes in monotypic species is emphasized. The occurrence of recent hybridization and recent assumption of the unisexual habit in these species is shown to question the probable existence of a sex completely homozygous to sex in any of these species. Since the problem therefore becomes one of getting the abnormal chromosome complement associated with the same sex generation after generation, the disposition of the supposedly homozygous sex in the selective ability of its gametes is advanced as a better explanation of the facts. A more thorough knowledge of the cytological behavior of unisexual species and their related hermaphroditic species and hybrids makes the theory of sex chromosomes in plants illogical.

Remarks Concerning A Natural History Survey of Great Smoky Mountains National Park. ARTHUR STUPKA.

The Great Smoky Mountains National Park, established in 1926, is an area of approximately 420,000 acres lying astride the high crest of the Southern Appalachian Mountains. The North Carolina-Tennessee state line runs the full length of the park for a distance of 71 miles, dividing the total area equally between these two states. Sixteen peaks have elevations in excess of 6,000 feet.

The region is one of very ancient rocks, and its vegetation has remained undisturbed for some 200 million years. The climate is diversified, with relatively cool and humid conditions prevailing at high altitudes. As in all national parks, no hunting, trapping or picking of plants is allowed; collecting permits, however, may be granted to scientific workers.

In order that park visitors may better understand and enjoy the natural features which distinguish this area an educational program is soon to be offered as a free government service. Guided field trips, lectures, exhibits and publications will be the means whereby visitors may better acquaint themselves with the park. The securing of information on which this program is to be based has been our problem since 1935, and in this the park naturalist has been assisted by various scientific workers. Certain members of the North Carolina Academy of Science may be interested in assisting with the many floral and faunal problems which remain.

The Distribution of the Fiber Population on the Cotton Seed. JERRY H. MOORE.

The distribution of the fiber population on the seed coat has been measured in the following types of cotton:

Naked seed[1] (no fuzz on the seed coat)

Semi-naked seed[1] (fuzz mostly on the micropylar and chalazal ends of seed)

Fuzzy seed[1] (dense fuzz population on the seed coat)

Tufted seed[2] (dense fuzz population on the micropylar end of the seed)

The results indicate that the distribution of the fiber population (commercial hairs or long fiber) of the several types studied is similar; because the population of each type is densest at and near the chalazal region of the seed and becomes less dense in passing from there toward the micropylar end; and also because the density of population decreases in going outward in a horizontal direction toward the raphe from a line on the surface of the seed coat directly back of the raphe. While the distribution of the fiber population of the types is somewhat similar, comparisons made for Goodness of Fit for fiber distribution indicate that the fit for the fuzzy- and naked-seed types is fair and that the agreement for fuzzy and semi-naked types is poor.

The relation of variability in the density of fiber population on seeds of the fuzzy type to fiber length and also to fiber weight per inch has been determined. The simple correlation of density of population with fiber length is not significant, while the simple correlation of density of population with fiber weight is significant with a negative association, that is, an increasing density of fiber population is associated with a decreasing fiber weight. Both of these relationships are probably affected by the position of the fibers on the seed coat.

Archaeology in North Carolina. JOFFRE L. COE.

Archaeology is a discipline that draws knowledge from every field of science and organizes it to make a comprehensive study of man in the past. It seeks to extend the horizon of recorded history, to examine and evaluate the product of man's achievement, and to obtain an objective understanding of the conquest of civilization.

Before Sir Walter Raleigh dreamed of a new England or the child Virginia was born to Eleanor Dare there were many different tribes of

[1] *Gossypium hirsutum.*

[2] *Gossypium barbadense.*

Indians occupying the geographic area we now call North Carolina. Their history is long and colorful, and may, in part, still be read with the aid of archaeological methods.

In the past North Carolina has shown little interest in its prehistory and has allowed a large part of its aboriginal monuments to be destroyed by erosion, plowing, and promiscuous digging, but now the efforts of the North Carolina Archaeological Society are being rewarded in that more and more people in the state are becoming aware that archaeology is a legitimate tool with which we may supplement the recorded history in our books and manuscripts.

Mechanical Analysis of Triassic Sediment. WILLARD BERRY.

Eighty samples were collected from twenty-one localities in the Triassic of Anson County, North Carolina Twenty grams of each sample were agitated with water for twenty-four hours, then the silt washed off and both silt and coarser residue dried. The coarser material was fractioned through 8, 16, 20, 30, 40, 50, 100, and 200 mesh United States series screens for ten minutes and all portions weighed and charted on a per cent basis. The fractions were given a preliminary examination for minerals, the following being identified—quartz, feldspar, coal, chlorite, muscorite and biotite; in addition, several as yet unidentified minerals were observed. The prevailing chocolate red of the sediments is due to iron staining of the quartz, which it is possible to wash clear. Discarding the samples which did not break down, we have fifty-seven which it is possible to group into nine shales or silt stones and eight arkosic sandstones and conglomerates on the basis of the mechanical analysis charts. At the present time, I cannot definitely correlate these beds on this basis as the petrographic examination is not completed and more samples must be collected, but the conglomerates and shales show a tendency to line up with a more or less NE-SW strike which corresponds with field evidence. Additional work will confirm or disprove the value of mechanical analysis plus petrographic examination in correlating Triassic sediments.

Vitamin A in Sheep's Milk and Colostrum. R. E. CLEGG AND G. HOWARD SATTERFIELD.

A study of the variation of the vitamin A content of the colostrum and the milk of ewes and the effect of mature and immature soy beans on the vitamin A content of the milk and colostrum was investigated.

The vitamin A content was determined by the reaction of Carr and

Price and was estimated in a Lovibond tintometer. The carotene content of the milk and colostrum was also estimated in the tintometer.

The vitamin A content of the later milk averaged 0.51 blue units per gram of sample. The vitamin A concentration of the colostrum was found to be from 5 to 57 times as great as in the later milk. The group fed on mature soy bean hay was lowest in average vitamin A content, while the group fed the immature soy bean hay was the highest. Under the conditions of the experiment the carotene content of the later milk was higher than that of the colostrum. On the average the colostrum of the older animals contained more vitamin A than did the colostrum of the younger animals.

Studies of the Fruiting Habit of the Peanut. G. K. MIDDLETON AND P. H. HARVEY.

By tagging peanut (*Arachis hypogaea*) flowers as they appeared, their distribution on the plant and the development of the fruits were studied on three varieties. Well matured seed developed largely from flowers produced in the first three or four leaf axils. Most immature seed and unfilled pods, or "pops," also occurred in this same relative position, but there was a slight tendency for the latter to occur further out on the branches Under the conditions of the experiment, Virginia Bunch (large seeded variety) produced a relatively large number of "pops" and few good seed as compared with the North Carolina Runner (small seeded variety) which produced few "pops." Jumbo Runner was intermediate in this respect.

The Production of New Species of Cotton. J. O. BEASLEY.

The chromosome number was doubled in 12 types of cotton by immersing apical meristems for 24 hours in 0.2 per cent solution of colchicine. Since a number of the types were sterile hybrids, doubling their chromosome numbers gave fertile polyploids, some of which are probably new species, for they are expected to breed almost true. The new types of cottons have fiber characteristics not possessed by the previously existing cottons; there are possibilities that some of these polyploids or their derivatives will produce fibers suitable for special purposes.

Polyploidy makes it possible for plant breeders to transfer the wealth of genes in distantly related wild species to cultivated types. Since certain combinations of species give sterile polyploids because the species are too closely related, it is proposed that relationships can probably be changed with the use of x-rays.

The Manner in Which an Excess of Carbon Dioxide Reduces the Intake of Water by Plants. PAUL J. KRAMER.

Saturating the water or soil in which plants were growing with CO_2 reduced the transpiration rate within one half to one hour by from 34% to 51%. The rate of exudation from the stumps of detopped plants was reduced by over 70%. The rate of water movement through living root systems attached to a vacuum pump was reduced by 38% to 56% or approximately as much as the rate of transpiration. While the percentage reduction in exudation was high the amount of water supplied to the plant by physiological absorption, which is the type of absorption responsible for exudation, is only about ten per cent of the total required by a freely transpiring plant. The complete cessation of physiological absorption would not explain the large reduction in absorption by transpiring plants. It is believed that increased resistance to water movement across the living cells of the cortex is the most important cause of reduced absorption This increased resistance probably results from changes in the colloidal structure of the protoplasm and cell membranes brought about by low pH.

The Cytology of a New Species of the Plasmodiophoraceae. ALMA J. WHIFFEN.

In all of the genera of the Plasmodiophoraceae studied cytologically, reduction division has been reported as occurring just previous to the formation of the resting spores. There are two views as to where in the life cycle karyogamy occurs. It is believed by Cook that the zoospores fuse in pairs, the nuclei of the plasmodium are diploid and the akaryote stage and reduction division occur previous to both the formation of resting spores and of zoosporangia. Horne and Webb contend that the zoospores do not fuse but that fusion occurs during the akaryote stage and the nuclei of the plasmodium are haploid. In *Octomyxa Achlyae* the akaryote stage and reduction division do not occur in the plasmodium that gives rise to zoosporangia. All nuclear divisions in the zoosporangia are of the typical mitotic type. The nuclei of the plasmodium which gives rise to resting spores pass through the akaryote stage and undergo reduction division. The chromatin of each daughter nucleus of the reduction division is divided on a quadripolar spindle, giving rise to a tetrad of daughter nuclei. At the end of the tetrad division the protoplasm is cut up into uninucleate resting spores which are aggregated in groups of eight. In *Octomyxa Achlyae* fusion is believed to occur during the akaryote stage. The quadripolar spindle is unique among the fungi.

Vegetative Regeneration in the West Coast Manzanitas. J. E. ADAMS.

The Manzanitas, members of the ericaceous genus *Arctostaphylos*, are a conspicuous and often dominant element in the chaparral of the Sierra Nevada and Coast Ranges of California. Taxonomically the genus is a difficult one owing to the slight degree of differentiation in flowers, fruits, and foliage. Workers without field experience are thus likely to recognize few specific heads. In the field, however, the species are observed to be different in their reaction to fire. Some are killed outright by fires of low intensity while others are able promptly to establish a new crown by sprouting from the base. This reaction to fire does not cut across specific lines. The specific reaction of the Manzanitas to fire was first observed by Jepson in 1916 and in a recent revision of the genus has been given considerable taxonomic weight. Although the ability to regenerate by sprouting after destruction of the top by fire or mutilation is not unique, *Arctostaphylos* offers a striking case of sharp specific differences in this respect. Of thirty-three species recognized about one-third are sprouters.

Those species which sprout have a conspicuously enlarged, usually globose root-crown while those that are killed outright by fire have no such enlargement. Under the influence of recurring fires the enlarged root-crowns may by rapid lateral extension become large and heavy woody platforms or tabular structures 6 to 8 feet in diameter from which sprouts arise in great profusion following fire.

Although fire may influence to some degree the size, shape, and rapidity of development of the enlarged root-crown in the sprouting species, it is not responsible for its existence as has been heretofore supposed. Even in very young seedlings the beginnings of the enlarged root-crown are observable as a small nodule which increases regularly in size as the plant grows. In plants growing in areas unvisited by fire this enlarged root-crown may attain a diameter of 1 or 2 feet.

The difference in regenerative ability of the species is of considerable importance in the construction and maintenance of fire-breaks in chaparral and timber land and in flood and erosion control. The taxonomic usefulness of this feature is greatly enhanced by the fact that the enlarged root-crown is a normal structure readily observable at any stage of development.

Peat Formation in the Carolina Bays. MURRAY F. BUELL.

The Carolina Bays, elliptical depressions on the coastal plain which are believed to have been formed by a shower of meteorites, were

originally occupied by lakes. Doubtless many of these have disappeared through erosion; some have been drained and are completely under cultivation; a few still contain lakes; but most of them are completely filled with peat. The way these peat deposits have been built up is illustrated by the present lakes where peat formation is still going on. Island formation through the establishment of trees and shrubs on the bases and knees of pioneer cypresses together with a shrub mat growing out slowly from the margin contribute organic debris which falls into the lake and accumulates as peat. In these lakes herbaceous vegetation is scarce. This together with the pioneer role of trees makes a striking contrast with northern bog lakes.

A New Species of Trematode, Telorchis caudata, from Chelydra serpentina. H. GRADY BRITT.

Specimens 2.04 to 4.2 mm. long by 0.3 to 0.62 mm. wide. Acetabulum located at end of first fourth of body length; 0.105 to 0.178 mm. in greatest diameter. Oral sucker slightly oval with diameters from 0.178 by 0.19 mm. to 0.24 by 0.26 mm. Short prepharynx. Pharynx broadly oval, 0.129 to 0.84 mm. long and 0.153 to 0.123 mm. broad. Short esophagus measuring 81 microns. Ovary spherical or broadly oval, right of median line, located at beginning of second third of body length; greatest diameter 0.15 to 0.23 mm. Seminal receptacle present. Vitellaria begin at level of ovary and occupy middle third of body. Eggs 14.8 by 32.0 microns in region of ovary. Testes in contact, posterior testis the larger, 0.186 to 0.34 mm. long by 0.21 to 0.33 mm. wide. Anterior testis 0.2 to 0.34 mm. wide by 0.16 to 0.24 mm. long. Genital pore one-half to two-thirds the distance from acetabulum to crural fork. Cirrus sac extends from genital pore to below anterior margin of ovary. Excretory system Y-shaped. Host: *Chelydra serpentina*.

Telorchis caudata nov. sp. resembles *Telorchis stenonura* Ingles more closely than any other North American species. It differs from that species, however, in several respects. *T. caudata* is smaller, oral sucker and pharynx larger, ratio of acetabulum and oral sucker different, esophagus shorter, position of genital pore farther from acetabulum, eggs larger, host different, and vitellaria begin at different level of ovary.

The Openings of the Seminal Receptacles in the Earthworm. ELTON C. COCKE.

The exact location of the openings of the *seminal receptacles* of the

earthworm is incorrectly described in many old and present day text books on zoology and general biology. These openings are almost always described or pictured as communicating to the exterior through pores on the "*ventral side*" of the worm. This is not the case at all for they actually open well up on the *lateral* sides about in line with the lateral setae.

There are possibly two reasons why this error has gotten into the texts and from them become well established in the minds of teachers and students, these are: (1) in dissecting earthworms they are usually opened along the mid-dorsal line and the sides pinned down flat, then the *seminal receptacles* are seen in a plane with the nerve cord and other ventrally located structures; (2) it seems logical from the very nature of the process of copulation that these pores should be on the ventral side in line with the male genital pores and seminal groove.

Careful observation with a lens or dissecting microscope will reveal the openings of the *seminal receptacles* about in line with the lateral setae. Cross sections of the worms through these organs will further convince one that the openings are on the *lateral sides* and not on the "*ventral side*" as has been so often described.

Note on a Sampling Restriction. JOSEPH A. GREENWOOD.

It is known that the number of samples selected must be pre-assigned or determined independently of the results. In certain types of testing adaptable to an ordered selecting of samples such as the die tossing type, this pre-assignment of length of experiment constitutes a real restriction. This paper injects an element of choice in an otherwise tight procedure. With the samples consecutively grouped in blocks, the (restricted) sizes and an upper bound to the number of blocks given in advance, the probability that a deviation ratio of $+X$ or better be attained in the total at any of the block division points is shown to be less than $p + (n - 1)p'$. p is the probability of attaining it on block one, p' that of failing on block one but succeeding on block two. n is the upper bound to the number of blocks. A brief table of p' is also given.

On Loci Associated with Osculants and Penosculants of a Plane Curve. E. A. CAMERON.

At a typical point M of a plane curve C, which satisfies certain general conditions, there exists a unique member of a specified class of curves φ, which has maximum order of contact with C at M. This member of φ, designated as Γ, is called the osculating member of φ to C. There

also exists at M a one-parameter family of curves of class φ which have contact with C of one lower order than does Γ. This family, designated as g, is called a family of penosculants. Γ may have associated with it a point P of such a character that the points corresponding to P for the members of g form a continuous curve L. The relation between the envelope of L and the locus of P, as M moves along C, is studied. A general principle describing this relationship is stated and illustrated. The statement and several illustrations of a dual principle are also given.

Conditions on Two Singular Matrices A and B Such that AB and BA May Have the Same Reduced Characteristic Function. H. V. PARK.

The Reduced Characteristic Function of a matrix M in the complex field is that polynomial, $\varphi(\lambda)$, of lowest degree such that $\varphi(M) = 0$. It can be shown that $\varphi(\lambda) = \frac{|M - \lambda I|}{\theta(\lambda)}$, where $\theta(\lambda)$ is the g.c.d. of all n^2 first minors of the determinant $|M - \lambda I|$.

Let $X = AB$, $Y = BA$, $x(\lambda) =$ R.C.F. of X, and $y(\lambda) =$ R.C.F. of Y. If A and B are singular then $x(\lambda)$ is not always equal to $y(\lambda)$. The purpose of this paper was to obtain necessary and sufficient conditions that $x(\lambda) = y(\lambda)$ if both A and B are singular.

The following most important results were obtained:

THEOREM 1: A necessary and sufficient condition that $x(\lambda) = y(\lambda)$ is that there exist integers $\alpha > 0$, $\xi \geqslant 0$, such that the matrices $(AB)^j$, $(BA)^j$ are both of rank $> \xi$ for $j < \alpha$, while they are both of rank equal to ξ for every $j \geqslant \alpha$.

THEOREM 2: If A is of rank r, a sufficient condition that $x(\lambda) = y(\lambda)$ is that ABA be of rank r.

THEOREM 3: If $r \leq 2$ and if $ABA \neq 0$ when $r = 2$, then $x(\lambda) = y(\lambda)$ if and only if AB is of the same rank as BA.

THEOREM 4: If $n \leq 3$, then $x(\lambda) = y(\lambda)$ if and only if AB is of the same rank as BA.

THEOREM 5: If $g(\lambda)$ is the R.C.F. of Z, a necessary and sufficient condition that $x(\lambda) = y(\lambda)$ is that the two matrices X and Y both *do* or both *do not* satisfy $g(\lambda) = 0$.

The matrix Z in Theorem 5 is obtained from $Q^{-1}BP^{-1} = \begin{pmatrix} U & V \\ W & Z \end{pmatrix}$, where P and Q are non-singular matrices chosen so that

$$PAQ = \begin{pmatrix} O & O \\ O & I_r \end{pmatrix}.$$

Conditions that a Matrix B be Expressible as a Polynomial in a Set of Partial Idempotent and Nilpotent Elements of a Matrix A. H. M. NAHIKIAN.

In this investigation, we assume a given nth order matrix $A = (a_{ij})$ with elements a_{ij} in the field of all complex numbers. Assuming that A possesses partial idempotent and nilpotent elements φ_{ij}, η_{ij}, we seek conditions on a matrix B that it be expressible as a polynomial $B = \Sigma a_{ijk}(\varphi_{ij}\eta_{ij})^k$ in these elements, where the a_{ijk} are scalars. We conclude that:

(1) It is necessary that B permute with A.

(2) The scalars a_{ijk} in the expression $B = \Sigma a_{ijk}(\varphi_{ij}\eta_{ij})^k$ are unique.

(3) If A has elementary divisors $(\lambda - \alpha)^{e_i}$, $e_1 > \cdots > e_r$ then $B = \Sigma a_{ijk}(\varphi_{ij}\eta_{ij})^k$ has a unique canonical form M, where $P^{-1}BP = M$, and $P^{-1}AP = A$.

(4) If A is a scalar matrix, then every matrix B is expressible as a polynomial in a set of partial elements of A, either irreducible or pseudo-partial.

(5) If A has elementary divisors $(\lambda - \alpha)^{e_i}$, and if $AB = BA$, a sufficient condition is that the factors $(\lambda - \lambda_1)^{e_1}, \cdots, (\lambda - \lambda_r)^{e_r}$ in the characteristic function $f(\lambda) = \prod_{i=1}^{r} (\lambda - \lambda_i)^{e_i}$ of B be relatively prime in pairs.

(6) If A has elementary divisors $(\lambda - \alpha_i)^{e_{ij}}$, a necessary condition is that B have elementary divisors $(\lambda - \lambda_i)^{\nu_{ij}}$, where $\nu_{ij} \leq e_{ij}$.

(7) A necessary condition is that A and B both be expressible as polynomials in a third matrix C.

(8) If A and B are expressible as polynomials $A = f(C)$, $B = g(C)$ in a third matrix C, then B is not necessarily expressible as a polynomial in a set of partial elements of A unless $f'(\gamma_{ij}) \neq 0$, where γ_{ij} are the characteristic roots of C.

(9) A necessary condition is that B satisfy a matric equation

$$B^\nu + F_1(A)B^{\nu-1} + \cdots + F_\nu(A) = 0,$$

where ν does not exceed the number of elementary divisors of A, and where the $F_k(A)$ are scalar polynomials in A.

The Relation Between the Reading and the Area of Exposure of a Photronic Foot Candle Meter. J. B. DERIEUX.

A source of light was placed so that its rays fell on the sensitive surface

at normal incidence, and so far away from it that the rays were practically parallel. In the first method used, 'an iris diaphragm was placed over the sensitive surface, which was circular, and concentric with it, then opening the diaphragm in steps the instrument reading of foot candles was observed, the diameter of the diaphragm aperture measured, and the area which it exposed computed. The graph, with area exposed, and foot candle reading, as axes, was a smooth curve but far from being straight, as it should have been if the reading was proportional to the area. Rising from the origin, it curved away from the area axis as the area was increased, thus indicating that the effectiveness of the area increased toward the periphery. And at the center, the reading was zero up to an area of about 0.5 square centimeters, thus indicating a dead spot there. The graph of the ratio of reading to area, and area, as axes, was a smooth curve with increasing ratio as the area increased, and concave toward the area axis. The ratio had a value of zero up to .5 square centimeter and a value 5 for an area of 6 square centimeters.

A test as to effectiveness of different parts of the periphery, and the center, was made by placing a 1 square centimeter aperture at different points and observing the reading. At right, top, left, bottom, and center, the readings were approximately 4, 6, 5, and 3, and 2 respectively. This showing that different parts near the periphery were not equally effective, and that the center was less effective, as was found by the previously described method.

The effectiveness of different sectors, each of which included a portion of the center and of the periphery, was measured. This was accomplished by cutting a 45 degree sector out of a metal disk which was of the same diameter as the cell head, and rotating in 45 degree steps. The reading again showed a variation, which was in accord with the readings with the aperture along the periphery.

The conclusions are that the readings are not proportional to the area. In the first test, where the area was increased concentrically with the cell, and the area was found to be less effective near the center, it was first thought that this was due entirely to the larger resistance resulting from the greater path of the current from that area, since the electric contacts are at the periphery. But when the further investigation found the variations in the sensitivity of the periphery, and of the sectors, it was finally concluded that the variation was produced by the variation in the sensitivity of different areas of the cell.

The Production of a Uniform Magnetic Field in a Long Rectangular Box. RUSSELL H. LYDDANE AND ARTHUR E. RUARK.

Extending work by F. K. Harris, we show how to produce a highly uniform magnetic field in a rectangular box long compared with its cross section, by using a pair of rectangular coils placed on opposite sides of the box. If the width of each coil is 1.73 times the distance between them, the region in which the field deviates less than one per cent from the value at the center is about 9 times larger than that obtained with previous coils having other width-distance ratios.

Use of square wire-bundles causes the main correction terms depending on finite cross section to vanish. The homogeneity achieved compares favorably with that obtainable by Helmholtz coils.

On the Variations in Wave Form at Different Points in the Tank Circuit of a H.F. Vacuum Tube Oscillator. SHERWOOD GITHENS, JR.

It has been customary, in construction and mathematical treatment, to view the operation of a high frequency vacuum tube oscillator as an entity, and to assume that the time-variation of the current and the magnetic field is the same at every point in the circuit. Observations made upon the h.f. magnetic field in the tank circuit inductance reveal that the field's time-variation differs from point to point; and that it is truly sinusoidal at only one point, and then only under certain conditions. Therefore, the "purity" or "harmonic content" of the output from the oscillator depends quite markedly upon the location of the output coil with reference to the primary, or tank, coil. The data show that balancing of the primary circuit and proper placing of the output coil are perhaps more potent means of improving emitted wave form than the use of wave-traps to selectively repress undesired frequencies.

H. L. BLOMQUIST,
Secretary.

PROCEEDINGS OF THE ELISHA MITCHELL SCIENTIFIC SOCIETY

OCTOBER 11, 1938 TO MAY 9, 1939

386TH MEETING, OCTOBER 11, 1938

J. W. HUDDLE: *The Paleontologist's View of Species.*

It was reported that Dr. MacPherson who had been elected vice-president at the 385th meeting could not serve, and the president appointed a committee consisting of T. F. Hickerson, chairman, F. K. Cameron, and W. F Prouty, to appoint a vice-president. Dr. E. A. Cameron was chosen for this office.

At this meeting further arrangements were made for the luncheon to be given on October 24 by the Mitchell Society to the National Academy, which was to be meeting here at that time.

387TH MEETING, NOVEMBER 8, 1938

ARCHIBALD HENDERSON. *Unfamiliar Methods of Constructing the Roots of the Quartic Equation.*

Archibald Henderson gave three methods which may be described as novel as they are apparently not to be found in the literature on the geometric solution of the quartic equation. In each of these methods, use is made of the parabola $x^2 = y$, of which, for class-room use, a templet should be available.

The first method, based on Ferrari's solution of the quartic, proceeds in the usual manner until the Resolvent Cubic is found. By a translation in the x-direction, the second term of the Resolvent Cubic is removed. By the use of the templet and a certain circle, the roots of the Resolvent Cubic may be determined geometrically (three if all the roots are real, otherwise one). Setting each of the quadratics (three pairs in all), arising from three roots, equal to y, we construct the resulting three pairs of parabolas. These parabolas intersect the x-axis, giving rise in the abscissas to the roots of the quartic equation; and in three ways (or one), in the following groupings: $(x_1, x_2; x_3, x_4)$, $(x_1, x_3; x_2, x_4)$, $(x_1, x_4; x_2, x_3)$.

The second method, by use of the substitution $x^2 = y$, transforms the

quartic into a conic. The four roots of the quartic are then the abscissas of the four points of intersection of this conic with the parabola $x^2 = y$.

The third method likewise employs the conic and the parabola, used in the former method. The necessary and sufficient condition, that the one-parameter family of conics on the four intersections of conic and parabola degenerates into a pair of straight lines, proves to be the familiar Resolvent Cubic of Ferrarri's solution (see method one). If this cubic have only one real root, this gives rise to one pair of lines, which intersect the parabola $x^2 = y$ in four points, the abscissas of which are the roots of the quartic. If, however, the three roots are all real, the parabola may be dispensed with, the intersections of any two pairs of lines with each other, but not among themselves, are the four points whose abscissas are the roots of the quartic.

Exceptional cases may be satisfactorily handled.

(Note: The constructions given above are natural consequences of two papers by Archibald Henderson (the second in collaboration with A. W. Hobbs) in the American Mathematical Monthly **35**: 337–348, 1928, and **37**: 515–521, 1930.

E. C. MARKHAM: *The Decomposition of Ammonium-deuterium Chloride.*

A letter addressed to the Society by Fred E. Wright, home secretary of the National Academy of Sciences, was read, expressing in resolution the appreciation of the Academy for the Mitchell Society luncheon tendered the members of the Academy at the autumn meeting.

The Permanent Secretary read an expression of appreciation, on behalf of the Society, to Dr. R. E. Coker for his excellent work as chairman of the local committee on arrangements for the autumn meeting of the National Academy.

388TH MEETING, DECEMBER 13, 1938

L. D. BURLING: *Some Results Obtained with the Prototype of a New Geophysical Instrument.*

The first public announcement of the application of the statistical method to a new type of radiation. Begun in 1932, the study has involved the making of well over one hundred thousand observations, in laboratory and field, upon radiations which appear to depart from known radiations widely enough to warrant their introductory description.

The radiations seem, in general, to be of two kinds: one which scatters

in all directions and diminishes rapidly in intensity with distance, and another, very penetrative, which seems to be confined within certain directions. Fifteen chemical elements and several mineral compounds have been studied. Both types of radiation were given off in all cases, but only the penetrative rays have been subjected to a rigorous qualitative and quantitative survey.

The penetrative radiations from the sources studied prove to be severally characteristic, distinguishable the one from the other, and the instrument used in their analysis can be so adjusted as to effect a radio-tuning-like selection from among them. This permits sole entry into the instrument, at any one time, of the particular radiations desired. In its present form, however, the resolving power of the instrument enables the operator easily to make a separation only between elements which belong in different columns of the periodic table. That elements within the same column are relatively hard to separate, and that certain elements and the minerals with which they are usually in intimate association exhibit even greater approximation of tuning rate, have been the subjects of collateral paragenetic study.

Linearly, and at the same time vertically, disposed sources have proven, both in the laboratory and in the field, that the penetrative radiations from each of the elements have a characteristic compass direction or azimuth. The respective azimuths vary locally, or with the magnetic declination, or for undetermined causes, but they do not change their relative position with reference to the meridian. And the sequence, counting clockwise from the meridian, arranges the azimuths of the elements studied in order of their atomic weight. For magnesium, lowest in atomic weight of the elements studied, the easterly deviation is approximately forty-five degrees.

In the southwest quadrant, and there counting clockwise from due south, occur correlatable azimuthal traces for each of the elements, also in order of atomic weight. Here, however, the traces are more closely approximated, and the angular diversion of each, from the meridian, is less than that of the comparable trace in the northeast quadrant. Viewed from above, the entire azimuthal trace, for any one vertical linear source, is thus bent at a point directly above or coinciding with that source.

For compounds, the azimuthal trace is apparently a linear resultant; if the molecule is firmly knit (oil). It appears to be multiple if the molecule is rock-salt-like.

Experimentally controlled spherical or point sources have been lowered to depths of 1500 feet, and with the source at suitably disposed levels within this range the behavior of the radiations has been studied. First, it may be stated that quantity readings through one hundred feet and through fifteen hundred feet of solid rock and water vary by so little as to suggest that the difference between radiations so penetrative and those to which the literature has already introduced us may be one involving a new energy level. Second, and perhaps more important, is the fact that the radiations from a point source lie within the azimuthal plane discovered for a linear source of identical composition, but that they are not equally distributed within that plane. They are gathered into bundles of radiation which crudely resemble, in diversity of intensity and spacing, the light and dark banding in a line spectrum. And, in the spacing and intensity of these beacon-like bundles, each element seems to have its own characteristic pattern. It may be announced, also, that the inclination of the individual bundles varies with the depth of the source, increasing from three minutes of arc per hundred feet of rise, below one thousand feet, to thirty-five minutes of arc per hundred feet of rise, in the two hundred feet immediately below the ground surface.

Subsurface study of adjacent greatly disproportionate veins of gold ore has shown that the radiation from the smaller of the two actually extends outward toward the greater, and decreases in intensity in that direction. The minimum is much closer to the smaller vein than it would be if the larger vein were not so large, but a minimum is there And in the shadow of the smaller vein there will be found no radiation from the larger vein, even though the smaller is far within the usual range of radiations from the large neighbor.

Further details of the behavior of the radiations themselves, the fact that they can be interpreted in terms of both wave and quantum theory, and something of the uses to which the radiations have been put in geophysical studies of local and regional underground structure and of mineral and ore deposits in ten of the States, were included in this first report of progress of an investigation which has been characterized through the years by ever increasing accuracy, dependability and range of application.

E. K. PLYLER: *Infra-red Absorption of Certain Liquids.*

389TH MEETING, JANUARY 10, 1939

H. W. BROWN: *Recent Advances in our Knowledge of Influenza.*

Official announcement was made of the death on January 4th, 1939, of President H. V. Wilson. Vice-President E. A. Cameron occupied the chair.

390TH MEETING, FEBRUARY 14, 1939

G. C. KYKER: *Determination of Quinine in Malarial Blood.*

R. J. WHERRY: *Factorial Analysis of Affective Report and Concomitant Physiological Changes.*

Classification of affective behavior has long been a matter of academic dispute. An attempt to approach a solution through the use of factor analysis was made through the use of Thurstone's centroid method on some data reported by Barmack. Four groups of subjects each working under slightly different conditions and each group having 84 readings for each variable were available. All groups contained data for length of work period, percentage of time spent in daydreaming, and position on the continua of interested-bored, pleased-irritated, fatigued-peppy, strained-relaxed, inattentive-attentive, and wide awake-sleepy. Two of the groups provided data for oxygen consumption, while the remaining groups had been measured for systolic and diastolic blood pressure and heart rate.

The factorial analysis yielded three factors. The most clearly defined factor was best interpreted as a pleasantness-pain continuum with high loadings for ratings of peppy, interested, pleased, attentive, wide awake, and relaxed. This factor seemed sufficient to explain such concepts as boredom, mental fatigue, interest, and attention since these factors received negligible loadings on the other factors.

The second factor was best interpreted in topological terms as a looseness-tension factor, the looseness end of the continuum being highly associated with daydreaming.

The third factor seemed to be a purely physiological strain-relaxation factor since it had the most consistent loadings on the physiological variables and practically negligible loadings on the verbal report data with the exception of partially accounting for the reports of strain-relaxation.

There was high consistency in the loadings from group to group, and the loadings on the psychological report variables seemed to be equally reliable to those on the physiological variables. The average of the average deviations of the factor loadings was about equal to the quantity $1/\sqrt{N}$.

Further research along this line was indicated, but little promise

was held for the finding of reliable physiological correlates which will differentiate discrete affective complexes.

391ST MEETING, MARCH 21, 1939

T. F. HICKERSON: *Damage Incurred by the Hurricane and Flood in 1938 in New England.* Illustrated by lantern.

392ND MEETING, APRIL 11, 1939

B. W. WELLS of State College: *The Plant Ecology of the North Carolina Coast.* Illustrated.

393RD MEETING, MAY 9, 1939

O. K. RICE: *Thermal Gaseous Explosions.*
J. B. FISK: *A Problem in the Artificial Transmutation of Elements.*

The following officers were elected for the year 1939–1940:

President—English Bagby.
Vice-President—E. L. Mackie.
Recording Secretary-Treasurer—J. E. Adams.

The permanent secretary, E. T. Browne, and the editors of the Journal, W. C. Coker, Otto Stuhlman, and C. D. Beers, were continued in office.

A resolutions committee consisting of R. E. Coker, Chairman, Archibald Henderson, and Wm. deB. MacNider, presented the following resolution which was unanimously adopted by the society.

HENRY VAN PETERS WILSON

The Elisha Mitchell Scientific Society makes this record of its great loss in the passing of its recent President, Henry Van Peters Wilson, on the fourth day of January of this year. Joining the Mitchell Society in its infancy, Dr. Wilson has for nearly half a century played a part second to none in its development as an organization of colleagues, friends and contributors to the advancement of science through research. His connection with the Society began soon after his arrival in Chapel Hill as Professor of Biology in 1891. At first as a collaborator with a small group of investigators, several of whom were charter members of the Society, and ever after as a teammate with successive generations of members, he has for 47 years been unremittingly devoted to its interests and strongly confident in the usefulness and capacity of the Society as a means of scientific expression and of stimulation to

better and more effective research. He was President of the Society in 1905–06 and was elected President again for the year 1938–39, which included the last months of his life. He served as a member of the Publications Committee from 1929–1937.

It was not, however, in any official capacity that Dr. Wilson rendered his greatest service to the Mitchell Society. It was rather as an exceptionally regular attendant, as an original contributor of papers that were models both for scientific significance and for technique of presentation, as a sympathetic and stimulating commentator upon the papers of others, as a general leader of discussion and as a personal exemplar of the spirit of research. It was in such capacities that he gave life and strength to our meetings.

We knew him also as a member of our University community continually and staunchly upholding the highest standards of industry and thoroughness, of accuracy and intellectual integrity; we esteemed him as a friend and companion, ready with sympathy, encouragement, and counsel of the best sort, and as one who combined a rare degree of clarity of thought with strength of conviction and incisiveness of expression. We enjoyed association with him as a conversationalist with catholicity of taste and capacity for illuminating comment on a diversity of topics of scientific or general interest. We admired him as a scientific leader who combined high distinction in achievement with simplicity of manner and modesty of bearing.

The Elisha Mitchell Scientific Society spreads this record upon its Minutes, directs that it be published in the Journal of the Society and orders that copies be sent to the members of his family in token of our appreciation of the leadership we have lost and of our deep sympathy for those who were nearest to him.

MATHEMATICS AND THE SCIENCES*

By J. W. Lasley, Jr.

INTRODUCTION

At the outset I wish to express my very sincere appreciation for the evidence of trust on your part which makes this occasion possible for me. It is quite a surprise when a group of scientists so honor a teacher of mathematics, for it is a moot question as to whether mathematics is a science. It is more than a surprise when that teacher is your speaker, whose association with science has been more that of a worshipper from afar than he likes to have to admit.

The duties of this office resolve themselves in large part to the retiring address which brings us here tonight. Upon asking myself what I might say to you that might in part compensate you for coming here, I thought it pertinent to consider with you the relation between mathematics and the sciences. With this purpose in mind I asked a philosopher colleague what he considered that relation to be. His reply was quick and pointed. "There is no relation", he said, "science thinks a thing in terms of other things; mathematics thinks a thing in terms of itself." His inference was that the two are mutually exclusive. This was very discouraging.

The history of science, however, does not seem to bear out the philosopher's contention. Until the time of Galileo (1600) that history is practically a history of mathematics. Although we have some knowledge of perhaps 6000 years of mankind's intellectual activity, we search in vain for any trace of science before 2500 years ago. True we have the pyramids, some 5000 years old, and their structure indicates the employment of scientific ideas. We have, too, the Rhind papyrus, 3500 years extant, and within its pages a kind of mathematical science. But the first scientist to emerge from the mists of antiquity was Thales, the mathematician of 2500 years ago. Almost contemporary with him is Pythagoras, a strange mixture of scientist and pseudo-scientist. Two centuries later came Democritus with the beginnings of an atomic theory

* Retiring address of the president of the North Carolina Academy of Science, Wake Forest, N. C., May 5th, 1939.

which even the opposition of an Aristotle could not down. Another century brings us to Euclid, but we must wait yet another century until, around 200 B.C., appeared that resplendent figure of old, Archimedes, bringing with him the law of buoyancy, the principle of the lever, the discovery of light reflection and the cry of "Eureka", which Whitehead says should be celebrated as the awakening cry of mathematical physics.

In the 17 centuries from that day to the time of Copernicus (1500) physics was to remain at practically a standstill. In another century these latent stirrings of the scientific spirit were brought to light for the first time in the father of modern science, Galileo Galilei, whom we know so well that we invariably call him by his first name. Important it is that he should be the first to formulate inertia, to discover the law of falling bodies, to invent the pendulum and the telescope, to discover the four satellites of Mercury and the sun-spots. More important still, says Millikan, that he should see "that force is proportional not to motion, but to the rate of change of motion, an idea the most profound in human thought." I dwell on Galileo because he is generally regarded as the originator of the modern viewpoint in science He, asserts Einstein, "saw that all knowledge of reality starts from experience and ends in it." Thus, as Whitehead so aptly puts it, "the world waited 1800 years from Archimedes to Galileo for someone who could relate abstract mathematical ideas to experimental investigation of natural phenomena."

Modern scientific inquiry as such seems to have begun with Roger Bacon in the 13th century. Leonardo da Vinci was indeed a voice crying in the scientific wilderness of the 15th century. Tycho Brahe's tables of 1601 were a first step in scientific observation. By means of them one could tell the position of the planets. It took a Kepler (1610) to see in them the three fundamental laws of planetary motion. Kepler could then tell us where the planets would be. In three inches he condensed the voluminous tables of Brahe, a tremendoüs scientific advance.

And then came Newton. Whitehead says that science came of age that day with Newton in his garden Einstein regards Newton's laws of motion as expressed in differential equations as the "greatest advance in thought that a single individual has even been privileged to make." He says further that Newton was the first creator of a comprehensive, workable system of theoretical physics. This one man, he continues, "gave intellectual guidance to science for 200 years." Perhaps no man, then or since, has known Newton's scientific view-point as has his modern prototype, Albert Einstein, who has done more than any man to

supplement his work. He says of Newton, "He believed that the basic laws and concepts of his system could be derived from experience. This is the meaning of '*hypotheses non fingo*'. Newton was uncomfortable about absolute space, absolute rest and action at a distance, since he found no basis for them in experience. The successes of his theories prevented discovery of the fictitious character of his foundations."

Daniel Bernouli (1700) following shortly after Newton has been called the founder of mathematical physics.

From this era dates the origin of organic chemistry. Lavoisier (1743–1794) transmuted alchemy into a rational science. Perhaps, as his judges said when he faced the guillotine, the republic had "no need of savants". Certain it is that chemistry had great need of Lavoisier.

Mathematics through the calculus as we know it today was shaped largely by the hand of Euler (1707).

Sedgwick and Tyler state in their history of science that at the beginning of the 19th century general physics and chemistry were "still in the preliminary stage of collecting and coordinating data, with attempts at quantitative interpretation, while in their train the natural sciences were following somewhat haltingly". Geike adds that at this time "geology and biology were not yet inductive sciences".

But the stirrings of science in the 18th century projected themselves into the 19th. Dalton (1803) with his law of multiple proportions for the formation of compounds supplied the first scientific approach to the atomic theory.

Lyell with his publication of his "Principles" in 1830 raised geology to the dignity of a science.

Biology was admitted to the union of sciences in the Victorian age through the efforts of Darwin, Spencer, Huxley, Wallace, and others.

By 1850 the older universities had founded scientific schools. Academies of science began to be formed. The public by the opening of the 20th century was science-minded. A new era was about to dawn.

This new era took the form of a new conception as to the structure of matter. There were significant undercurrents in the world of physics. Sir Humphrey Davy made what he called his greatest discovery, Michael Faraday. Faraday (1791–1867) discovered the principle of magneto-electricity, and originated the electro-magnetic field theory. The world was little aware of these tremendous happenings. Even at a time when the Atlantic cable was in operation Gladstone could (and did) ask Faraday whether electricity had a use. And Faraday replied, "Why, Sir, there is every probability that you will soon be able to tax it."

In 1850 Maxwell placed a mathematical support under Faraday's theories, to be followed by the experimental verification of Hertz.

Joule (1818–1889) found a mechanical equivalent for heat, namely, energy, giving the world the first law of thermodynamics.

Plank gave a description of radiation as incapable of emission in aught but units, the quanta. In this quantum theory fractions of a unit of energy simply do not exist.

De Broglie and Schrodinger combined the energy theory of Einstein with the quantum theory of Plank and compelled the joint wave-particle view of the atom. (Since then the physicist has been accused of teaching the wave theory on Monday, Wednesday, and Friday, and the particle theory on Tuesday, Thursday, and Saturday.)

Heisenberg proclaimed the doctrine that nature abhors not a vacuum so much as it does accuracy and precision.

Dirac extended the uncertainty principle of Heisenberg to the entire realm of atomic physics.

Pauli furnished us with his exclusion principle.

Millikan and Cameron gave us cosmic radiation.

Minkowski offered his space-time world.

Einstein supplemented the Newtonian mechanics, proclaimed the invariance of natural laws in inertial systems, the constancy of the velocity of light, the abandonment of simultaneity, the identity of mass and energy, claimed absolute motion incapable of detection, related time and motion, connected space and matter. Gravitation, that most elusive of concepts, appeared as the curvature, or crumpling, of a space-time continuum. But the electro-magnetic fields were not expressed in the field equations of general relativity. Later came a field theory in which gravitation and electromagnetic radiation were welded together. Only the expression of the atomic structure in terms of the field theory was, and still is, missing.

Here we are, and what a long way we have come. Let us examine some of the high and low places along the path. Let us see again something of the view from a few of the peaks and depression of the way. Let us inquire of Mathematics, the guide in this long and fascinating journey.

CONTINUITY

Perhaps we never realize its subtlety until we really try to find out the meaning of continuity. The writer of radio script uses the term to refer to his product. We have heard his programs. Can such an idea

be hedged about with difficulty? As is so often the case, an understanding of the concept implies an understanding of its opposite. The opposite of the continuous is the discrete.

Long ago there lived an excellent gentleman named Zeno. It was back in the time of the Pythagoreans, 500 years before Christ. This Zeno saw the conflict between these opposites, and used what he saw to deny the possibility of motion, to discourage placing bets on Achilles in his historic race with the tortoise, and for other strange and bewildering purposes. Even today one doesn't just rush in to show where Zeno was wrong. In his antinomies are found the baffling ideas of the infinite, the deceiving implications of continual divisibility. Down the years we trace these difficulties like a colored skein in the pattern of scientific thought. They face the scientist in his effort to understand the constitution of matter. Is this paper from which I read smooth and unbroken, or is it made of discrete particles bounding about hither and yon—a veritable bee-hive? The physicist leans to the latter view. (This opinion may help explain the nature of what is being read from these pages.) What, then, about action at a distance? How are light, radiation, energy, gravitation conveyed from here to there? What? No ether? Can we have ether without continuity? If the ether is a jelly-like mass, is it not composed of particles? If it is composed of particles, will not the quantum behavior of matter nullify the continuity of the action? If we have a continuous exciting cause, is it not strange that energy should emerge in units (quanta), or not at all? Are there no fractions? The physicist say, "No, no fractions." De Vries claimed that evolution proceeds by "explosions." But Darwin, Newton, Kant, Leibniz all believed in continuity. Plank's quantum theory replaces a continuum of states in an isolated system by a finite number of discrete states.

The mathematician has been through—I should say, is in this same turmoil. He has never fully recovered from the Pythagorean shock of the irrational. For a time it was thought that Weierstrass, Dedekind, and Cantor had laid the spectre, but the contrary views of Knonecker, Brouwer, and Weyl have to be taken into account. The attack of Bishop Berkeley on the calculus of Leibniz and Newton, the feeling that this calculus is making "bricks without straw," must at least have a hearing. The critics of continuity claim that nothing which can not actually be constructed by a finite number of steps can hope to lead to a discipline free of paradox. They maintain that all analysis must eventually subject itself to the domination of the positive integer.

Among the non-mathematical scienticists who share this view Karl Pearson states the position thus, "no scientist has the right to use things unless their existence can be demonstrated."

Some may meet these difficulties by what has been called "a continuous but discreet silence."

Certain it is that a Thomas Wolfe may write "Of Time and the River" with a much more glib assurance than may an Einstein.

Simple things these—in Time such a perfect continuity; in number such discreteness (I came near saying "discretion"), and in the shadows an infinity trying to bridge the gap.

CAUSATION

Jeans maintains that the "steady onward flow of time is the essence of the cause and effect relation" It is but natural, then, that when continuity is in question, causation should take its place under the microscope of scientific scrutiny. There is more at issue than the mere *post hoc ergo propter hoc* argument. We are so used to drawing inferences from data, that it is hard to realize on what flimsy grounds many of our conclusions rest It is hard, too, to see how we may do intellectual business at all without the ability to infer effect from cause. The great 17th century of Galileo and Newton encouraged the scientist to think of causation as something on which he could definitely rely. Modern physics takes the position, so ably formulated by Pearson, that causation is intelligible only in the perceptual sphere as "antecedence in a routine of sense impressions." With the precision of measurement in studying natural phenomena came the realization of the statistical character of those measurements. Into the relations connecting the numbers arising in this way began to enter questions of doubt. The descriptions of the phenomena exhibited by the relations were seen to be more exact than the uncertainty of the data warranted. It began to appear that the descriptions described little more than what Weyl has called "statistical regularities." Pearson has put it thus bluntly, "In the order of perceptions no inherent necessity can be demontrated . . . necessity has a meaning in the field of logic, but not in the universe of perception . . . causation is neither a logical necessity, nor an actual experience."

This position seems at first glance to be at variance with the "if this, then that" of mathematical disciplines. The causation which inheres in logic, whose presence we so naively hope for in our scientific thinking, seems actually to emerge in the tenets of the mathematician. How,

then, may the scientist fit data patently statistical in character into mathematical form, clearly non-statistical in character. If, as Pearson claims, "contingency and correlation replace causation in science," how does the mathematical equation tell us a true story of natural phenomena? Pearson answers this in part by saying, "contingency is expressed in a table with cell-dots forming a band. This band viewed through an inverted telescope gives a curve. This curve is the mathematical function."

In the language of the mathematician, the scientific relation approaches the mathematical formulation asymptotically. Perhaps a more nearly correct statement is that both the scientific data and the mathematical description near each other in a process of successive approximation which would warm the heart of a Poincare.

Although many scientists feel, with Jeans, that the advent of Plank's quantum mechanics has dethroned continuity and causation, they in large measure share his belief that the appeal to a purely statistical basis may be a cloak for ignorance and that cause and effect of an unknown character may actually be in operation.

DETERMINISM

One would expect that questions about cause and effect should have philosophical implications. There arise the old questions of determinism and freedom. Determinism, according to Dantzig, "consists of the assumption that, given any natural phenomenon, the various features that characterize it are completely determined by its antecedents. The present knowledge permits prediction of the future course." "Each extension of the law of causation," says Jeans, "makes belief in freedom more difficult." Pearson claims that "our belief in determinism is the result of supposing *sameness* instead of *likeness* in phenomena. Eddington asserts that "physics is no longer pledged to a scheme of deterministic law." When asked why one magnet repels another, Whitney replied, "By the will of God," and added "science can enslave us, or it can make us free, but it we who make the choice." Others hold the view that our bodies and our minds are as physical as inert matter, made of the same chemical elements to be found in the remote stars, subject to the same inevitable laws; that the same determinism which holds for them holds also for us. Compton, speaking at the University last November, refuted the claim that man's actions depend on physical law. But he claimed it a vital question for science to find out whether man's actions are determined; and if so, by what factors. He main-

tained that it is no longer justifiable to use physical law as evidence against freedom.

Into this confused picture comes mathematics with its law of averages and its probability theory. Almost within the last decade the uncertainties of the situation have been amplified by Heisenberg into an Uncertainty Principle, which says, "To any mechanical quantity Q there corresponds another quantity P in such a way that the product of the uncertainties in our knowledge of Q and of P can never be less than a certain constant h, Plank's constant; hence the more accurately we determine Q, the more ignorant we are of P." In the Newtonian mechanics a knowledge of the position and of the velocity of an electron at an instant determines the future position of that electron, but Heisenberg assures us that we can never know both. The more accurately we determine the position, the less accurately we know the velocity; and vice versa. This concept of uncertainty seems to put the *coup de grace* on determinism. But who shall say that the very law of averages which replaces determinism may not itself be as great a despot as the dictator which it displaces? May there not be still a new determinism dominated by probability, just as there may be a new causation whose source is unknown to us? Or shall we, with Compton, and others, align ourselves with freedom because, as he says, "I find reason to believe in freedom, and wish to find whether such freedom is consistent with the recognized laws of physics." It would be a fine irony, indeed, if science, the greatest liberator of men's minds, denied to itself that freedom which it has so unstintingly given to mankind.

LAW

This outlook brings us to consider our ideas of law anew. When we say law, what do we mean? Do we think of brass buttons and a uniform? Do we think of statutes to which as citizens we owe obedience? Do we think of natural law, such as Newton's universal law of gravitation, or of mathematical law, such as Gauss' law of quadratic reciprocity, or of the philosopher's definition: "Law is Unity in structure difference"?

Weyl tells us that the mathematical lawfulness of nature "is a revelation of Divine reason." "The world," he says, "is not a chaos, but a cosmos harmoniously ordered by inviolable mathematical laws." We speak of Boyle's law for a perfect gas, of Kepler's three laws of planetary motion, of Dalton's law of multiple proportions and many, many other laws. The scientist maintains that his chief concern is the discovery of nature's laws. Just what does he mean by that? Is civil

law one thing; and natural law another? Does law mean one thing to some of us, and quite another thing to others of us? Or, is there a philosophic pattern behind all law?

In trying to understand the world we live in we observe and we experiment. We assume the validity of sense perception. We assume that normal human beings observe and experiment in much the same way. If we have ever listened to witnesses testify in court, we know just how much of an assumption that is. And furthermore, what, pray, is a normal human being? Many of us feel that all the knowledge that we obtain of natural phenomena comes through the senses, despite Pearson's continued insistence that in thinking we deal not only with sense impressions but with stored up opinions of former sense impressions. We measure with all the uncertainties attendant thereto; we think, or try to, amid all the doubts above mentioned as to continuity and causation and determinism weighing upon us. How in this atmosphere can we get at law?

The mathematician stands serene in this confusion. To him law is simply the matter of an invariant under a set of transformations. This invariant incorporates the unity, if any, present in the differences of structure in the situation in question. This unity answers the question as to how we may see the permanent in the transitory. The scope of it tells us how we may see the general in what is particular.

In civil law we have to make the statute. Whether we like it or not, that statute may be broken. Still in the changing pattern of civil law the statute formulates what unity is possible in the diversity of action which it seeks to control. In natural law these differences in action take the form of the great dissimilarities in observed phenomena. The unity is the common part, if such there be. The natural law expresses this unity amid the action differences. Its form is never final until it partakes of the form of the mathematical invariant.

POSTULATION

Reflections on the nature of law bring forcibly to our minds the postulational character of our thinking. We do well to examine the meaning of our most fundamental concepts as well as the lines of argument leading to our most important conclusions. Every science has its undefined terms. Aught else is an infinite regression. When analysis fails, we rely on the properties of our concept to define it for us. In setting up the discipline for a science, some of the propositions must be accepted without proof for similar reasons. The criterion for choice is

simplicity. This is not as simple as the name indicates. By simplicity, as used here, is meant logical simplicity. As Einstein so aptly words it, "By 'simplest' we mean that system which contains fewest possible mutually independent postulates, or axioms." This attitude of modern science is far removed from Newton's *hypotheses non fingo*. It is an attitude undoubtedly provided by the mathematician Einstein continues, "nature is the realization of the simplest conceivable mathematical ideas. I am convinced that we can discover by means of purely mathematical constructions, the concepts and the laws connecting them with each other, which furnish the key to the understanding of natural phenomena. Experience may suggest the appropriate mathematical concepts, but they most certainly cannot be deduced from it. Experience remains, of course, the sole criterion of the physical utility of a mathematical construction. But the creative principle resides in mathematics."

Such a postulational approach to mathematical thinking was seen by Euclid in so far as our inability to define satisfactorily all our terms. The fact that even a mathematician cannot prove everything was not formulated until Pasch almost in our own time. Now the necessity of a postulational approach to both definitions and theorems is a universally accepted tenet of the mathematician.

SYMBOLISM

Whether we agree to this postulational so-called simplicity, we can have no doubt of the existence and efficacy of symbolism in both mathematics and the sciences. The desire of constructibility, so ably championed by Knonecker, has found its way into our search for an understanding of the nature of matter. We hear Lord Kelvin exclaim that he "can understand nothing of which he cannot make a mechanical model." To meet this desire we have the dynamic Rutherford-Bohr model of the atom and the static Lewis-Langmuir model. But we are told these are too simple and definite to be regarded as other than intellectual conveniences. The ether is symbolized for us as a jelly-like mass with remarkable properties. We are warned, however, that the universe is not completely picturable in a graphical sense. Radiation and gravitation elude such a mechanical description. We speak of particles and waves as describing the behavior of light and radiation, but we are reminded that the electron is only a symbol for convenience of speech. Eddington tells us that matter and all else in the physical world has been reduced to a "shadowy symbolism." When we ask what

do the symbols stand for, the reply is that it doesn't matter. (One is reminded of the story that is told of Professor Lefevre, of the University of Virginia. He is said to have greeted his philosophy class one fine morning with the startling pronouncement, "What is mind? No matter. What is matter? Never mind.") "Physics," continues Eddington, "has no means of probing beneath the symbolism. Nor does one have to understand the symbols. What we have to understand are the conditions to which the symbols are subjected." The symbols themselves are dummies. Any other would do as well.

The mathematician is thoroughly in accord with this use of symbolism. He has likened his subject to a game of chess. The rules of the game play the rôle of postulates. In such a game Bell tells us there is no question of "truth"; there is merely a question as to whether the rules have been complied with. To Hilbert mathematics is "a game played according to certain simple rules with meaningless marks on paper."

PREDICTION

We have heard of old that a prophet is not without honor. For the man in the street the ability of science to predict the future holds a particular fascination. He is thrilled by the story of an Adams and a Leverrier working apart each computing from the perturbing influences of an unknown source on Uranus, the position of a new planet, Neptune, just 52 minutes from where Galle later found it. He reads of the electromagnetic waves predicted by Faraday and Maxwell and verified by Hertz. He has heard of the more recent prediction by Einstein of the shift toward the red end of the spectrum, caused by the deflection of light in a gravitational field, verified in the solar eclipse of 1919. These and many others, such as Mendelejeff's prophecy as to the discovery of gallium, scandium and germanium, such as Hamilton's prediction of conical refraction, have cast science in the rôle of one of the major prophets. Even Pearson concedes science the ability to predict, as well as to describe.

Mathematics provides in its differential laws a pattern for these predictions. "A differential law," say Einstein, "tells us how the state of motion of a system gives rise to that which follows it in time." "If we know how the velocities and accelerations depends on position, we can trace out the past and future of our universe," says Pearson. This is done by means of differential equations with proper boundary value conditions. The chemist does it when he predicts the position of the electron in its orbit. The astronomer does it when he predicts the

position of the planet in its orbit around the sun. Despite Heisenberg's uncertainty as to our ability to measure both position and velocity, the schedules of the planets are much better known than are those of the crack Chicago to New York trains.

INVENTION

Science is known to many only for its inventions. Much of its popularity with the masses is due to the added comforts and enjoyments with which it supplies them. Their eye is open for the so-called practical things of science. The auto, the radio, and the thousands of gadgets which give us our arm chair civilization, endear science to the heart of the multitude.

But this has not been the path of scientific progress. These things have usually been but by-products. Hertz little thought when he verified Maxwell's electromagnetic waves that he was laying the foundation for radio. Perhaps as often the practical leads back into the fundamental principles, as do the principles lead to invention. Again, when the scientist thinks himself most theoretical, he may be near a very useful practicality. "Indeed," says Richards, "the developments of the wave mechanics now in progress may be fraught with graver practical consequences for humanity than the approaching commercialism of television or rapid trans-oceanic passenger flying."

It is an old story to the mathematician, whom the cry of "practicality" fails to arouse. Archimedes, tracing his conics in the sand when Marcellus' soldiers snuffed out his genius, had no thought of a Kepler using them to describe the paths of the planets. Argand, Gauss, Wessel in their abstract imaginings about the complex number little fancied that they would later in the hands of Maxwell place a firm footing under modern electrical theories. Riemann, Cayley, and Sylvester had no thought that they were preparing the way for Einstein. Sturm and Liouville had no concern for the wave mechanics of De Broglie, which their researches made possible. The meditations of Cayley appear in the modern theories of Heisenberg and Dirac. Fermat, Gauss, DeMoivre, Pascal could not possibly have foreseen that their probability theory would one day revolutionize physics. Indeed, the theory of today is so often the practice of tomorrow. If it were not, it would be no great matter. But, as Philip has said, "it is only against the background provided by the pure research of yesterday that the technical problems of today can be viewed in their proper setting and tackled with a reasonable prospect of success. Work in the pure sciences,

however remote from the practical issues of the moment, is building up a reserve of knowledge and technique for future workers to draw on."

COSMOGONY

One of the reasons why one studies mathematics and the sciences is this: To obtain a better understanding of the world in which he finds himself. As the sciences and mathematics have developed, so have developed our views of the cosmos. To primitive man who thought of himself at the center of the universe, to men who with Ptolemy regarded the earth as the centre, to men who with Copernicus placed the sun in this strategic position, the cosmos presented a very different view. This view colored many aspects of their thinking. It led to the formulation of a very different philosophy of life. So much so that someone has said "tell me a man's view of the Universe, and I will tell you what sort of man he is." There have been religious upheavals attendant upon man's change in his views of the world about him. In our own day his view is suffering what is perhaps its greatest change. Not only has the sun been displaced from its central position, but in its place nothing has been substituted. We are told that there is no known center; no reference frame in which to orient a path in the cosmos. We have mysterious cosmic rays beating down upon us from an unknown source with unknown effects. Out in an unknown place somewhere, Millikan suspects, cosmic radiation may be rebuilding matter—an inverse phenomenon never dreamed of until our time. These are tremendous disturbances in man's view of the cosmos. That there have been no attendant religious disturbances is a conspicuous testimonial to intellectual freedom. The lay world is becoming accustomed to regard almost as commonplace views which former generations held to be impossible, in some instances unintelligible. That the world can achieve this transition complacently is due in large part to the tough intellectual fiber provided by mathematics and the sciences.

SOCIAL IMPLICATION

Einstein asserts that "concern for man himself and his fate must always form the chief interest of all technical endeavors . . . in order that the creations of our minds shall be a blessing and not a curse to mankind." That scientific findings have the potentiality of becoming the latter is the thought of many at this time when modern warfare threatens the very existence of civilization. May not our very scientific endeavors prove a Frankenstein? It has even been suggested that science take a

holiday in order to let the rest of the world, particularly the world of good-will, catch up. But, as Hill has pointed out, the scientist is after all a human being. Can he know which of his discoveries will be put to harmful ends? Mankind must learn to take the good and the bad together. "It is ironical," says Gregory, "that greater productivity through invention should bring more distress and unemployment rather than an increase in human welfare." Social progress has not kept pace with scientific progress. Russell takes the position that if mankind were rational, his conquest of nature would increase his happiness and well-being. "Only kindness," he says, "can save the world, but even if we knew how to produce kindliness, we should not do so unless we were kindly."

This dilemma, many believe, is caused by our failure to apply to social and economic problems the same intelligent analysis that has been applied to scientific problems. They assert that scientific thinking is definitely on a plane above thinking in other fields—and that this explains the fact that science has outdistanced non-science. The ideal of thinking is presented in the perfectly welded chains of mathematical proofs. The sciences approximate this norm more closely than do the nonsciences. Social, as well as scientific progress, comes with the finding of truth. The pattern for the search for truth is mathematical thinking.

FAITH

One rarely thinks of faith as an element essential to the scientist. The scientist is by definition one who knows. What need then can he have of faith? Mark Twain says that, "Faith is believing what you know ain't so." Somewhere Hilaire Belloc exclaims, "Oh, one should never, never doubt what no one can be sure about." Does this levity contain some truth? Does not the worker with facts need faith as a sort of whistle to keep up his courage? If he is never really sure, does he not need faith to bolster up this insecurity? Or is it that a calm, pervading faith is one of the necessary tools in the kit of the scientist?

That the latter is the attitude with which the scientist should approach his task we are assured in the retiring address of President George D. Birkhoff, of the American Association for the Advancement of Science, delivered this past year at the Christmas meeting in Richmond. There one of America's foremost mathematicians spoke to America's scientists of the faith that is his. It is fitting that we here try to catch an overtone of that meeting.

Mr. Birkhoff claimed that whether it is the mathematician dealing with number, or the physicist with matter, the biologist with organism, the psychologist with mind, or the sociologist with social values, there is behind one and all an inherent faith guiding the reasoned superstructure which they create upon intuitional concepts. Whether it is the mathematician's belief in the existence of infinite classes, the physicist's belief in the presence of a discontinuous process at work in the theory of radiation, the biologist's belief in a vitalistic theory of life, the psychologist's belief in a physiological accompanyment to every psychical fact, or the sociologist's belief in societal progress, Birkhoff emphasizes faith as an "heuristically valuable, more general point of view, beyond reason, often in apparent contradiction, which the thinker regards as of supreme importance as he endeavors to give his conclusions the greatest possible scope."

Some think that there is an opprobrium attached to any belief, that belief and science are mutally exclusive. Do these same people believe in the processes of logic? Do they have faith in the rationality of the human mind, in the similarity of the perceptive and reasoning faculties of normal, civilized beings? Is it not in their code that nature is orderly, and that there are spiritual values underlying material facts?

CONCLUSION

In the foregoing we have traced in broad outline the advance in scientific thought from the earliest time down to the present. We have pictured the scientist journeying down this path with his guide, the mathematician. We have noted some of the scenes from certain plateaus and valleys in the path. Continuity, causation, determinism, law, the postulational method, symbolism, prediction, invention, cosmology, social implication, faith, have passed in review. We have endeavored to point out how the guiding hand of the mathematician has aided the traveler along the way. The physical aspects of science, particularly those relative to the structure of matter, have been stressed because they are better known and because of the major importance of matter as "the building blocks" of the universe. There has been no disposition to indulge in propaganda for mathematics. Mathematics needs no "sales talk" to the scientist. It has been rather an effort to understand its function in the domain of scientific thinking. This relation seems to be much like that of the guide to the mountain climber. Hardly could a guide be better fitted for his task. Bound to the traveler by a philosophical bond they rise or fall together. The assistance is by

no means all on one side. Many are the instances in which the problems of the scientist have enriched the theories of the mathematician. Many are the instances in which the theories of the mathematician have aided in the solution of the problems of the scientist. The equations of the mathematician are regarded by many as the only language which nature speaks. Helmholtz expressed this thought in the words, "the final aim of all natural science is to resolve itself into mathematics." Jeans has this in mind in his statement, "all the pictures which science draws of nature, and which alone seem capable of according with observational fact, are mathematical pictures . . . the Universe seems to have been designed by a pure mathematician." Even Galileo back in the beginning of what we are pleased to call modern science said, "Nature's great book is written in mathematical language." Whitehead maintains that the aim of scientific thought is, "to see what is general in what is particular and what is permanent in what is transitory." In this vision science utilizes the general abstraction of mathematics and adopts its theory of invariants. The concept of progressive change is basic in the study of natural phenomena. This same idea is the mud-sill of the calculus. "With the calculus as a key," continues Whitehead, "mathematics can be successfully applied to the explanation of the course of nature." When classical physics suffered the impact of the Michelson-Morley experiment it was forced by its own findings to reexamine its foundations. "In this emergency," to quote Dantzig, "it was entirely due to the flexible mental apparatus with which the mathematician supplied them, that the physical sciences have at all survived this drastic revision." Richards asserts that "when we reach the core of physical reality, the truth is presented in mathematical equations." Weyl claims in the long ago the Pythagoreans held that the world was not "a chaos, but a cosmos harmoniously ordered by invariable mathematical laws." Jeans expresses it in the words, "nature seems to know the rules of mathematics as the mathematicians have formulated them in their studies without drawing on experience of the outer world."

We come now to the end of tonight's account of this amazing journey of the scientist. In saying farewell to our scientific traveler we hear that insistent injunction from the mouth of his guide, so aptly put by Dantzig, "read your instruments and obey mathematics; for this is the whole duty of the scientist."

DEPARTMENT OF MATHEMATICS,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

STUDIES ON THE TURBELLARIAN FAUNA OF THE MOUNTAIN LAKE BIOLOGICAL STATION. I. ECOLOGY AND DISTRIBUTION

By Frederick F. Ferguson, M. A. Stirewalt, T. D. Brown, and W. J. Hayes, Jr.

With 12 Text-Figures and One Chart

During the latter part of the 1938 summer session of the Mountain Lake Biological Station, extensive collections were made in the streams, ponds, and springs of the region in order to list the Turbellaria.

Our object is not only to list for the convenience of future workers the turbellarian fauna thus far found in the Mountain Lake region, either by ourselves or others, but also to add our bit to the small amount of information now available treating of the ecology and geographical distribution of these forms.

Thirty-eight species and varieties of twenty-three genera were studied. Three investigators have published descriptions of one or more species of Triclads or Rhabdocoeles for the vicinity of the Mountain Lake Biological Station: Kenk, 1935; Gilbert, 1935; Nuttycombe and Waters, 1938.

The Mountain Lake Biological Station was established by the University of Virginia as a summer biological station in 1930. The station is about eight miles from Pembroke, in Giles County, Virginia, and about one mile from Mountain Lake. This lake is said to be the only natural lake in the southern Appalachians and one of the highest in the East. The station is at an elevation of approximately 4,000 feet on the divide between the Mississippi and Atlantic drainage areas.

Because of an abundance of rain and the presence of many springs and small mountain streams, it is an excellent collecting place. Because of the altitude factor, collections may be made representing life of both the Canadian and Austral Zones within a radius of five miles of the station.

Since the establishment of the Mountain Lake Biological Station by the University of Virginia, nineteen zoologists have worked on the Turbellaria in this region. Only three of these investigators have published material of an ecological nature on this particular region. The

others have, whenever possible, generously contributed notes for the preparation of this paper. The list of investigators includes Dr. William A. Kepner, Dr. Bruce D. Reynolds, Dr. Chauncey M. Gilbert, and Mr. Robert Brumfield of the University of Virginia; Dr. Paul R. Burch of Radford State Teachers College; Dr. Margaret Hess of Judson College; Dr. Ruffin Jones of William and Mary College (Norfolk); Dr. Trenton K. Ruebush of Yale University; Dr. Roman Kenk of the University of Puerto Rico; Dr. John W. Nuttycombe of the University of Georgia; Dr. Raymond L. Taylor of William and Mary College; Mr. Robert I. Bosman of Johns Hopkins University; Mr. Richard J. Porter of the University of Chicago; Col. Robert P. Carroll of Virginia Military Institute, and the authors.

TAXONOMIC LIST OF SPECIES REPORTED

Order 2: Rhabdocoelida
- Suborder 1: Notandropora
  - Family 1: Catenulidae
    - *Rhynchoscolex simplex* Leidy 1851.
    - *Catenula virginiana* Kepner and Carter 1930.
    - *Stenostomum arevaloi* Gieysztor 1931.
    - *Stenostomum grande* Child 1902.
    - *Stenostomum kepneri* Nuttycombe and Waters 1938.
    - *Stenostomum pegephylum* Nuttycombe and Waters 1938.
    - *Stenostomum saliens* Kepner and Carter 1931.
    - *Stenostomum tenuicaudatum* Graff 1911.
    - *Stenostomum tuberculosum* Nuttycombe and Waters 1938.
    - *Stenostomum ventronephrium* Nuttycombe 1932.
    - *Stenostomum virginianum* Nuttycombe 1931.
    - *Stenostomum* sp.
    - *Fuhrmannia* sp.
- Suborder 2: Opisthandropora
  - Family 2: Macrostomidae
    - *Macrostomum bulbostylum* Ferguson 1939
    - *Macrostomum riedeli* Ferguson 1939
    - *Macrostomum reynoldsi* Ferguson 1939
    - *Macrostomum ruebushi* Ferguson 1939
  - Family 3: Microstomidae
    - *Microstomum lineare* Schmidt 1848
- Suborder 3: Lecithophora
  - *Section* 1: Dalyellioida
  - Family 4: Provorticidae
    - *Provortex affinis* (Jensen) Graff 1882.
  - Family 5: Dalyelliidae
    - *Dalyellia* sp.
    - *Castrella truncata* Hofsten 1910.

Section 2: Typhloplanoida
Family 6: Typhloplanidae
Subfamily 2: Typhloplaninae
Typhloplana sp.
Castrada sp.
Subfamily 3: Rhynchomesostominae
Rhynchomesostoma rostratum (Müller) Luther 1904
Subfamily 4: Phaenocorinae
Phaenocora kepneri Gilbert 1935.
Phaenocora virginiana Gilbert 1935.
Subfamily 7: Mesostominae
Mesostoma sp.
Section 3: Kalyptorhynchia
Subsection 1: Eucalyptorhynchia
Family 18: Gyratricidae
Gyratrix hermaphroditus Ehrenberg 1831
Family 19: Polycystidae
Klattia virginiensis Kepner, Stirewalt, Ferguson 1939.
Order 3: Alloeocoela
Suborder 1: Lecithoepitheliata
Family 3: Prorhynchidae
Prorhynchus stagnalis Schultze 1851
Geocentrophora baltica (Kennel) Steinböck 1923*
Geocentrophora sp.
Suborder 3: Seriata
Family 11: Bothrioplanidae
Bothrioplana semperi Braun 1881.
Order 4: Tricladida
Family Planariidae
Curtisia foremani (Girard) Graff 1916.
Euplanaria trigrina (Girard) Kenk 1935.
Fonticola gracilis (Haldeman) Kenk 1935.
Fonticola morgani (Stevens and Boring) Kenk 1935.
Planaria dactyligera Kenk 1935.

STATIONS

Natural Lake

Mountain Lake (Fig. 1) is located between Salt Pond Mountain and Doe Mountain at an altitude of 3,873 feet. It is situated on what was for many years the main highway between Virginia and West Virginia. The interstate boundary line is about ten miles north of the lake. The first survey of this body of water was made in 1753 by Christopher Gist and is apparently valid today, the dimensions being about nine-tenths

* *Prorhynchus balticus* Kennel 1883.

Distribution Chart (Summer, 1938)

ANIMAL	STATION: Mountain Lake	Hoge's Pond	Farmer's Pond	Kessinger's Pond	McClarity's Pond	Evans Pond	V P I Pond I	V P I Pond II	'Lake Rana'	Water Supply Spring	Arsenic Spring	Twin Springs	Dividing Spring	Little Stony Creek	Mud Branch	Hunter's Branch	Sinking Creek	Big Stony Creek	Cascades and Bluffs	Little Cascades
Bothrioplana semperi	x																			
Catenula virginiana		z		y	x	y		x	x	x	y						x	x		
Castrada sp.	x																			
Castrella truncata																			x	
Curtisia foremani							x			y										
Dalyellia sp.	x						x			z	y								x	
Euplanaria tigrina	x								x	y										
Fonticola gracilis	x																			
Fonticola morgani	x									x				x						
Fuhrmannia sp.	x									x	x								x	
Geocentrophora baltica																			y	
Geocentrophora sp.										x										
Gyratrix hermaphroditus									x	y	z				x					
Klattia virginiensis						x	y													
Macrostomum bulbostylum			z				x													
Macrostomum riedeli											y									
Macrostomum reynoldsi														x					z	x
Macrostomum ruebushi							y													
Mesostoma sp.							x													
Microstomum lineare	y						x													
Phaenocora kepneri							x													
Phaenocora virginiana						y														
Planaria dactyligera	y																			
Prorhynchus stagnalis	y									y	x								x	
Provortex affinis										x										
Rhynchomesostoma rostratum	x																			
Rhynchoscolex simplex	x									x									x	x
Stenostomum arevaloi																			x	
Stenostomum grande	x	x					x													
Stenostomum kepneri	x															x			x	
Stenostomum pegephylum										x										
Stenostomum saliens	x																			
Stenostomum tenuicaudatum	x			x	x	x	x			x		x	x		x		x	x	x	
Stenostomum tuberculosum	x																			
Stenostomum ventronephrium																			x	
Stenostomum virginianum	x																		x	
Stenostomum sp.											x									
Typhloplana sp.	y							x												

x—few specimens; y—many specimens; z—very abundant specimens.

of a mile by about one-fourth of a mile. The surface is about one hundred acres and the greatest depth is about eighty-five feet. According to several geologists the lake is a natural solution collapse basin. The underlying formation is of Martinsburg Shale (Ordovician). This shale is very high in lime content. On the east and north sides of the lake, large boulders of hard sandstone (Clinton formation) (Silurian) have broken away from overlying formations and come to lie on the bottom of the lake so that they project from the water. The lake is supplied by several springs and a very abundant rainfall. The water is usually transparent to the extent that a standard Secchi disc may be

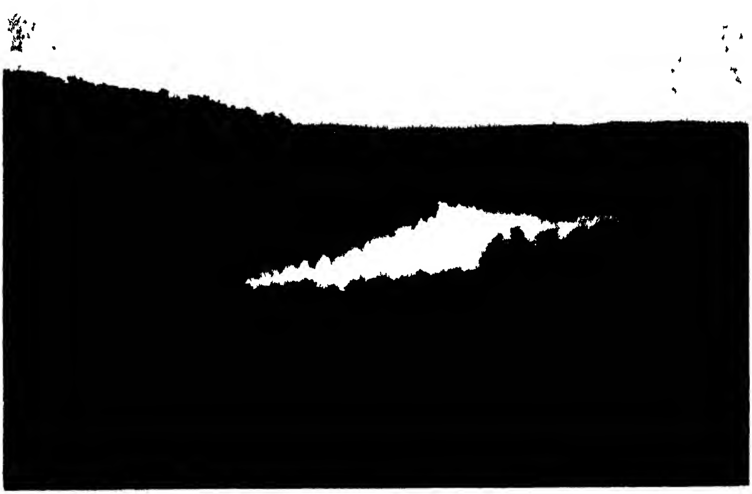


FIG. 1. Mountain Lake—looking northward

seen to the depth of eighteen feet. After very heavy rains the transparency may not be more than three or four feet. Analysis of the water shows the following solutes to be present as indicated in parts per million: mineral residue, 6.4; organic and volatile residue, 19.2; iron and aluminum oxides, none; bicarbonates (HCO_3), 30; free carbon dioxide (CO_2), 9; dissolved silica, 1.4; calcium, trace; magnesium, trace; nitrates, none. Dr. Edwin Powers and Miss Theresa Hickman, working in the summer of 1934, found the pH to change from 6.8 to 5.9 as the depth increased to seventy-five feet. They found that the oxygen content decreased from 5.32 to 5.15 cc. per liter between the surface and

a depth of 30 feet and decreased thereafter to 2.48 cc. per liter at a depth of 75 feet. In summer the temperature is slightly over 22°C at the surface. It decreases about one degree between the surface and the thermocline. Between about 15 feet and 30 feet of depth (the thermocline) the temperature drops rapidly from 21°C to 11°C and thereafter drops gradually to 9°C at the bottom. The physical and chemical properties of the water and mud of the lake are well described by Hutchinson and Pickford (1932). The water contains *Elodea*, *Isoetes*, *Chara*, unicellular and filamentous algae, and other plants. The lake shore is overgrown with evergreens mainly mountain laurel, rhododendron, and hemlock.

Collections were made at depths of from six inches to thirty feet. Study showed that the most abundant turbellarian fauna lived in the more shallow water, while only *Euplanaria* and *Rhynchomesostoma* were taken at the deepest point of collection.* Collections at the shallow southern end where several springs are found, proved to be the best. A more extensive study of depth collections will be made in the near future.

Artificial Ponds

Collections were made in several artificial ponds varying in length from twenty-five feet to four hundred yards.

Hoge's Pond (Fig. 2) is located in Giles County on U. S. Route 23, approximately two miles east of Pembroke, at an elevation of 2,035 feet, in a fairly rich limestone valley (Stone River formation) (Ordovician). This pond is about seventy-five yards long and not over eight feet deep. It is about sixty years old. It is fed by the overflow from a watering trough, which receives water piped from a mountain spring two miles away. The pond is bordered on different sides by pasture, cultivated land, and a patch of weeds and willow trees.

Farrier's Pond (Fig. 3), is on U. S. Route 23, two miles west of Newport. It is about two hundred yards long and its greatest depth is about 15 feet. A swampy place produced by a small mountain stream and including a large spring was dammed up more than eight years ago to form this pond. It is located at an elevation of 1,850 feet, on limestone soil (Stone River formation). The water contains large quantities of *Chara* and other algae. The greater part of the pond is unshaded, though there are several large willow trees at one end. The shore is in lawn grass.

* These depth studies were made by means of an automatic collecting dredge (Ekman dredge, Foerst Company).

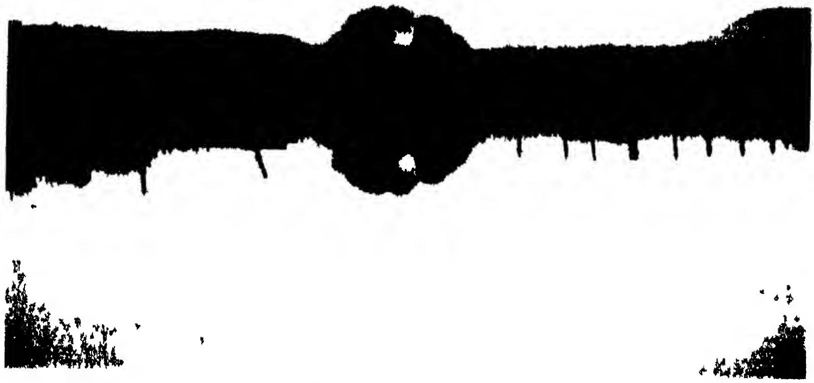


FIG 2 Hoge's Pond

FIG 3 Farrier's Pond

Kessinger's Pond (Fig. 4) was formed about twelve years ago by damming a small mountain stream in a swampy meadow. It is located in Giles County, Virginia, about two miles southwest of Newport at an

FIG. 4 Kessinger's Pond

FIG 5 McClarty's Pond

elevation of 2,100 feet. The pond is about fifty yards long and contains an abundant growth of green algae. Aquatic insects are very numerous. The surrounding soil (Martinsburg Shale) is in grass on one side, and wooded on the other.

McClarity's Pond (Fig. 5) is located between Kire and Interior, Giles County, Virginia, about two miles from Kire. It was formed about ten years ago by damming a small swamp fed by a mountain stream. The pond is more than fifty yards long and is ten feet at the deepest point. Plant and animal life is not abundant. *Juncus*-like plants are growing in the northern end of the pond. The entire pond is surrounded by grass-land. It is located at an altitude of 2,690 feet in Black Devonian Shale.

FIG. 6 Evan's Pond

Evan's Pond (Fig. 6), about two miles south of Blacksburg (between Blacksburg and Price's Fork), is apparently a small sink hole. It is located at an elevation of 2,200 feet in the Rome formation (Cambrian) It is fed by a small spring and has no outlet. Though the pond is only about fifteen yards long and only knee deep, local residents say that it has never been dry. The center of the pond which is in open water has a bottom composed of bluish gray mud. A lush growth of rushes and saw grass separates the open water from a weed-grown field.

On the campus of Virginia Polytechnic Institute, in Blacksburg, are

two artificial ponds. One, located between the Faculty Apartments and the University Club (Pond I) (Fig. 7), is about three hundred yards

FIG. 7 V P I Pond I

FIG 8 V P I Pond II

long. Pond I was formed about five years ago and contains abundant animal life and large quantities of *Spirogyra*. Pond II (Fig. 8) was formed about twenty-five years ago but was enlarged about a year ago

and contains almost no animal life but does contain abundant *Chara*. Both ponds are located at an elevation of 2,150 feet in Rome formation. Neither pond is shaded.

"Lake Rana" as it is known by the biologists of the Mountain Lake Station, is a small stream-fed pool on the grounds. It is a veritable catch-all for any aquatic plant or animal which may be brought for study. Many Turbellaria have been "planted" here for future study.

Springs

Collections were made in three large springs and many smaller springs on Salt Pond Mountain, Bear Cliff Mountain, Doe Mountain, and vicinity.

The Water Supply Spring of Mountain Lake Biological Station is located three-fourths of a mile southeast of the Station on Bear Cliff Mountain. It is at an elevation of 4,100 feet, and is located in Clinton formation (Silurian). The average summer temperature is 11°C. The spring is surrounded by mountain laurel and rhododendron. The border of the spring is over-grown with sphagnum moss. The bottom of the spring is covered with a layer of vegetable debris, principally dead leaves.

Arsenic Spring is a large spring on the banks of Big Stony Creek, two miles west of Kire, Virginia, between Interior and Kire. It is at an elevation of 2,689 feet in Black Shale (Devonian). This spring has proven very interesting because of its low summer temperature (11°C) and the large number of Turbellaria taken from it. The spring gushes from a bank, passes through a swampy area, and enters Big Stony Creek. It is in the swampy area of soft mud rich in organic matter and plant growth that the best collecting may be done. The spring and the swampy area are heavily shaded by deciduous trees.

Twin Springs are located one-half mile northwest of the Mountain Lake Biological Station, at an elevation of 3,680 feet. The springs form an alga-filled pool four yards long, surrounded by large hemlocks and mountain laurel.

On the road leading from the Biological Station toward the Virginia-West Virginia boundary line, approximately two miles from the Station, is Dividing Spring. This small spring is unique among those studied in that it has drainage partly to the Missippi and partly to the Atlantic coast. It is partially shaded and is filled with a rank growth of green algae.

Streams

Besides the many large and small springs, in the vicinity of the Biological Station, there are many typical, small mountain streams. Ex-

FIG. 9 Tributary stream to Little Stony Creek near Cascades

FIG. 10 Big Stony Creek

tensive collections were made in the small streams which unite with the Mountain Lake drainage to form Little Stony Creek. The streams

(Fig. 9) tributary to Little Stony Creek, from which collections were made, are called Mud Branch and Hunter's Branch. Collections were also made in Sinking Creek and Big Stony Creek (Fig. 10). Unlike the ponds whose locations have been pointed out, these streams are easily found by use of a map (U. S. Geological Survey topographical map, Dublin, Va.-W. Va. quadrangle).

FIG. 11. Little Cascades

Falls

Excellent collections were made in and near the cascades on Little Stony Creek. These are about four miles (six miles by road) west of the Lake. There are two falls – one called Little Cascades (Fig. 11) which has a twenty foot drop, and the second called The Cascades (Fig. 12) which has an eighty foot drop. They are located at an elevation of about 3,050 feet in the Clinton formation (Cacapon member) (Silurian). These cascades are surrounded by large hemlocks, rhododendrons, and deciduous trees. Excellent collections may be made by scraping the moss and algae from the rocks in the heavy spray of the falls. Surrounding the falls are high bluffs down which numerous spring-fed streams flow. These streams also offer good collecting places.

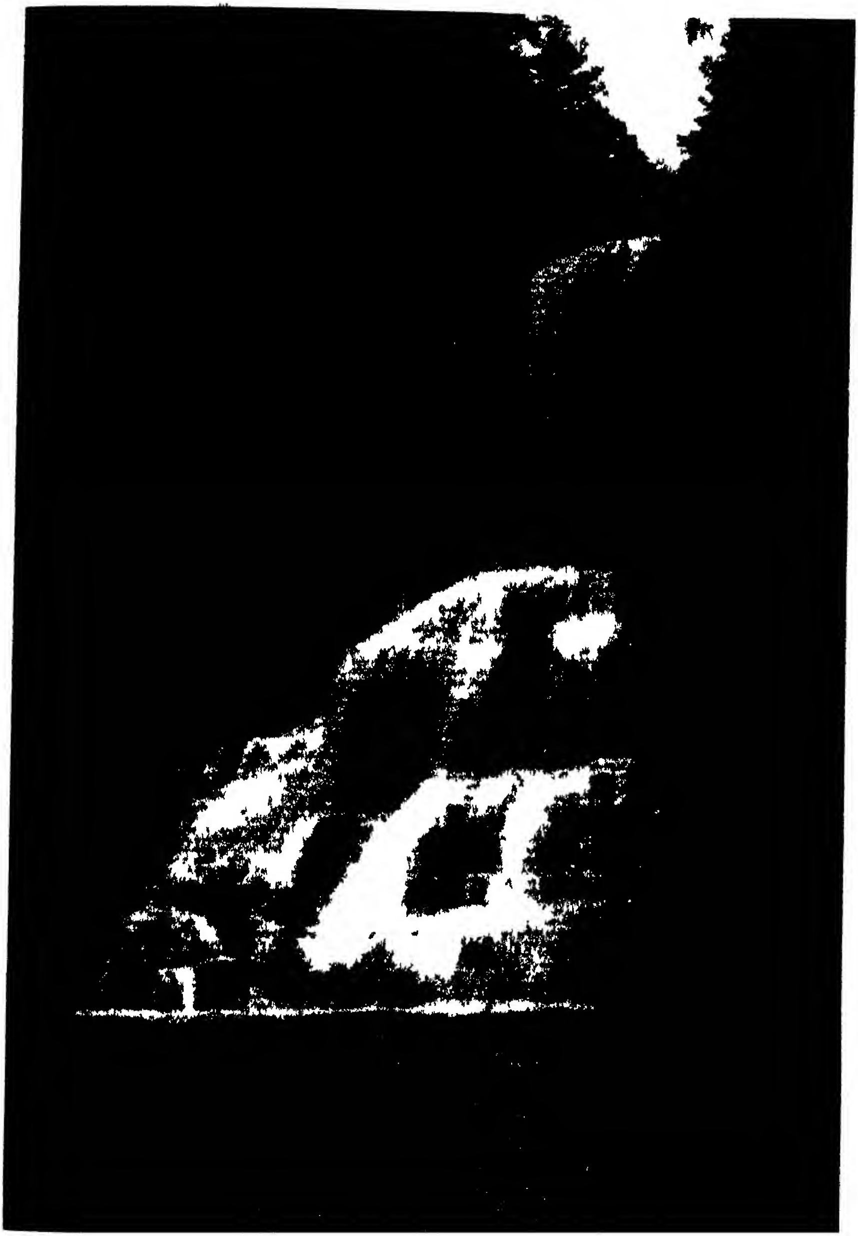


FIG. 12 The Cascades (Little Stony Creek)

DISCUSSION

The occurrence of *Macrostomum bulbostylum* to the exclusion of other Turbellaria in Farrier's Pond is of significance. This is a carnivorous, hardy species which is able to live under conditions which would destroy many other turbellarian forms. On the other hand, the pond is relatively new. We intend to "plant" several of the more hardy turbellarian species in Farrier's Pond in order to test whether only *Macrostomum bulbostylum* has been able to establish itself in the pond or whether it (or some other factor) has prevented other species from establishing themselves.

While the authors are cognizant of the fact that turbellarian species may not always be found in the same place from year to year, they are convinced that such a survey as this will aid in understanding the variable nature of these bodies of water. The Mountain Lake Biological Station presents an ideal environment for the completion of such an ecological study. A vast amount of such work awaits the students of Turbellaria here in America as well as elsewhere.

MILLER SCHOOL OF BIOLOGY,
UNIVERSITY OF VIRGINIA,
CHARLOTTESVILLE, VA.

LITERATURE

FERGUSON, F. F. A Monograph of the Genus *Macrostomum* O. Schmidt 1848. Part I. Zool. Anz. **126**: 7–20. 1939.

GILBERT, C. M. A comparative study of three new American species of the genus *Phaenocora* with especial reference to their reproductive organs and their relationships with other described forms of the genus. Acta Zool. **16**: 283–386. 1935.

GRAFF, L. v. Turbellaria. II. Rhabdocoelida. Das Tierreich, 35 Lief. 1913.

HUTCHINSON, G. E., AND G. E. PICKFORD. Limnological observations on Mountain Lake, Virginia. Int. Rev. Hydrobiol. **27**: 252–264. 1932.

KENK, R. Studies on Virginian Triclads. Journ. E. M. Sci. Soc. **51**: 79–125. 1935.

KEPNER, W. A., AND J. S. CARTER. *Catenula virginia* n. sp. Zool. Anz. **91**: 300–305. 1930.

KEPNER, W. A., AND J. S. CARTER. Ten well-defined new species of *Stenostomum*. Zool. Anz. **93**: 108–123. 1931.

KEPNER, W. A., M. A. STIREWALT, AND F. F. FERGUSON. *Klattia virginiensis* n. g., n. sp. Zool. Anz. **125**: 299–309. 1939.

KEPNER, W. A., E. D. MILLER, AND A. W. JONES. Observations upon *Rhynchomesostomum rostratum*. Zool. Anz. **107**: 188–192. 1934.

NUTTYCOMBE, J. W., AND A. J. WATERS. The American species of the Genus *Stenostomum*. Proc. Amer. Phil. Soc. **79**: 213-301. 1938.

RUEBUSH, T. K. The occurrence of *Provortex affinis* Jensen in the United States. Zool. Anz. **111**: 305-308. 1935.

STEINBÖCK, O. Monograph der Prorhynchidae (Turbellaria). Zeitschr. f. Morph. u. Ökol. **8**: 538-662. 1927.

LOCHETIC LUMINOUS DIPTEROUS LARVAE

By B. B. Fulton

Plate 27

While getting water from a mountain spring one night in early June, I noticed many steadily glowing specks of light scattered over the damp earth and rocks near the spring. The light had a bluish color unlike that of fire flies or their larvae. Returning to the spot later with a flash light, I discovered that the lights came from worm-like creatures living on webs strung across exposed hollow places in the damp earth or between rocks (Fig. 1). When disturbed they quickly glided along the web to conceal themselves in deeper parts of the cavities.

They had the general appearance of small Annelid worms but when examined more closely with a hand lens, a small but definitely insect type of head could be seen. I concluded that they were Dipterous larvae and attempted to rear adults but was not successful. Some months later I learned from the larval structure and habits that they must belong to the family Mycetophilidae or fungus gnats.[1]

Most of the larvae are about a centimeter or slightly more in length when extended and about .8 mm. in diameter. The segmentation is distinct only in the anterior part of the body. The integument has brown pigment giving an annulated appearance except about a fifth of the anterior end and the extreme posterior end which are unpigmented. Observed in the dark these parts are illuminated with a bluish light. Within the anterior part, two rows of dark bodies like loosely strung beads are visible.

When the larvae were removed from their habitat and placed on damp earth or moss in Petri dishes they proceeded to make new webs. The web is spun from the mouth and consists of a central strand suspended over a hollow place by alternating strands to right and left. There are generally several main strands leading to a deeper cavity where the larva lies concealed during the day. The whole web may be 4 or 5

[1] This finding was confirmed by Dr. O. A. Johannsen. Adult flies obtained in 1939 were identified as a new species of *Platyura* by Dr. Elizabeth G. Fisher, who also made the other determinations mentioned in footnotes.

cm. long and conforms to any available natural hollow. It may be spread vertically or horizontally but the branches are mainly in one plane except in the innermost cavity which may be filled with strands running in all directions. Most of the branches in the exposed portion are coated on the distal part with a whitish viscous material forming spindle-shaped bodies 2 or 3 mm. long which end abruptly about a half millimeter from the attachment of the strand. This material adheres to anything touching it but the remainder of the web is not adhesive. The larva rests on the flattened central strand, being held to it by a film of liquid which completely surrounds the body. It can glide rapidly forward or backward by means of peristaltic body waves, and can reverse its direction by doubling back on the same strand.

The web seemed to be well fitted for the catching of small prey. To test this I caught a small insect and by the aid of a flash light placed it in contact with a viscous spindle in one of the undisturbed webs by the spring. The fly larva immediately darted out of its retreat, and crawled over the insect coating it with liquid from the body, then grasped the insect with the mouth and pulled it back into the cavity. Examination of the webs by daylight revealed the remains of an ant and a small beetle. The most common insects in the vicinity of the webs were Collembola, which would be entirely consumed if caught. Termites were accepted as prey by the larvae confined in Petri dishes. The larvae remained concealed during the day but at night would come out far enough to expose the luminous anterior part of the body. Whether this light served to attract insects, is a problem I did not attempt to solve.

The spring where the larvae were found is located near Glenville in Jackson County, N. C., at an elevation of about 3500 feet. The spot is constantly moist and shaded. The larvae were most abundant in a partial cavern formed by an uprooted tree. A few others were found in the vicinity along a small stream under a rhododendron thicket, nesting both in soil and in rotten wood. Larvae were still present when I left Glenville on June 14, 1938. I visited the spot again by daylight on July 11, but could find no larvae. At night on the same date, I found a number of them at Highlands in a steep wet bank around a spring. None were ever found associated with fungi. The alimentary canals of preserved specimens examined later were found to contain only broken particles of small insects and mites. Living specimens were taken to Raleigh in Petri dishes both in June and July. One of the first lot reached the pupa stage and died, but all the others died in the larval

stage. The second lot was taken east during a hot period and all were found dead on arrival.

A survey of the literature on this group of flies failed to reveal any account of luminous or lochetic larvae inhabiting the United States. Wheeler (1) reviews the literature on the known webspinning lochetic species, a few of which are luminous. More recently Mansbridge (2) and Madwar (4) have described the habits of a number of webspinning English species, several of them from genera known by the adult to be represented in our fauna. Provided with this information about European species, I looked for Mycetophilid larvae in appropriate places about Raleigh during the past fall and winter. Several different species were found showing considerable diversity of habits, but none of them were luminous. They were kept in shell vials for further study.

One species, of which two specimens were obtained from cavities in rotten logs, spun sheets of web of extremely fine criscross strands. The web was invisible to the naked eye so that the larvae appeared to be suspended in mid air. The web was not adhesive and even small Collembola failed to become entangled in it. Although fungus growth from the original habitat was provided, the larvae were never observed feeding on it and both specimens died, apparently from starvation.

Another species[2] was found living gregariously in porous rotten wood. They filled the spaces in the wood with a network of rather coarse dry web. Small insects did not become entangled in the web but were apparently unable to crawl through it. The larvae fed on the rotten wood and matured on this food alone. With this species the web seemed to have no function except that of excluding predatory or parasitic enemies from the cavities in which they were feeding.

Three other species were found living in webs well adapted for the catching of small prey. The webs were several inches long and consisted of a main strand, more or less branched, suspended by numerous much branched smaller strands radiating in all directions (Fig. 2). The main strand was a thin slimy band on which the larva rested or was suspended under it. The side strands were fine threads loaded with small drops of clear liquid, like loosely strung beads.

One species[3] was found only in very rotten damp logs containing much fungus growth, either in old Passalus holes or on fungus growth on the under side of the log. The alimentary tract of one specimen was found to contain fine granular material with no trace of insect remains. The

[2] *Sciara* sp. near *jucunda* Johannsen.

[3] *Ceroplatus* (*Ceratolion*) new species.

species probably feeds largely on fungi. They were very slow to attack an insect caught in the web but would occasionally do so. Usually they would allow the prey to die in the web before feeding on it. The other two species[4] were sometimes found in cavities in rotten wood, but more often among damp dead leaves on the under side of rotten logs. They were usually not associated with fungus growth. They promptly attacked insects placed in the web.

When kept in shell vials with a piece of rotten wood or other absorbent material saturated with water, it was noticed that the size of the web droplets varied with the degree of saturation of the air. A high degree of humidity had to be maintained for optimum conditions. If the larvae were exposed to the air of a room, they would die in a few minutes. Mansbridge (2) found that the web droplets of Platyura would quickly kill a small Annelid worm by contact. Buston (3) found that this material contained oxalic acid and that a solution of oxalic acid of about the same concentration would also kill the worms. Dr. Ivan D. Jones tested the web fluid of my specimens and found that it also contained oxalic acid. No immediate toxic effect was observed with the termites and other insects used in feeding my specimens.

Aside from any benefit due to the oxalic acid the droplets adhere to small insects that come in contact with the web. Guided by the movement of the struggling insect, the fly larva glides toward the prey, keeping the rear part of the body on the main strand, with finger-like lobes on the terminal segment acting as an anchor. The mode of attack varies. Sometimes the larva will grasp the prey and drag it into the web destroying part of the web, but bringing the prey into contact with so many strands that it is rendered helpless. At other times the larva proceeds more cautiously and at first contact with the prey the body contracts so suddenly that a considerable amount of the liquid surrounding the body is deposited on or near the prey. This may be repeated a few times and then the larva crawls over the legs and jaws of the prey until the latter is so coated with slimy liquid that further movement is impossible.

On one occasion while looking for something to feed the larvae, I found some small spiders living in webs in a window. They suggested the proverbial relationship of the spider and the fly. Were these juvenile flies capable of reversing that situation? I selected one of the aggressive species, placed the spider in the end of the vial and prodded it into the web. Several of its legs came into contact with the web droplets and it began to struggle to free itself. The larva mobilized and

[4] *Platyura* sp., near *inops* Coquillett and *Platyura genualis* Johannsen.

came gliding along the main strand. The spider apparently saw the movement and ceased struggling. The larva was unable to locate the motionless spider and after a few minutes retired. The spider then resumed activity and the performance was repeated several times. Finally the larva continued to advance slowly and reached a leg of the spider. It grasped the leg and snapped back, jerking the spider farther into the web. On the next advance it crawled across the pedipalps and front legs and snapped back again leaving a slime deposit. Then it coated the remaining legs on each side and lastly crawled over the ventral side, grasped the tip of the abdomen and yanked it forward under the cephalothorax, leaving the spider in a helpless slime-coated ball.

A more highly developed webspinning ability is found in two other lochectic Mycetophilids. One was found by Cook (5) in Guatemalan caves. The web was suspended from the roof of the cave and consisted of a main horizontal strand over a foot long supported at intervals by vertical strands. Hanging from the horizontal strand were other vertical strands 1 to 3 mm. apart and 2 or 3 inches long, each with a viscous coating. The larva traveled on the horizontal strand and fed on small flying insects caught in the sticky fringe.

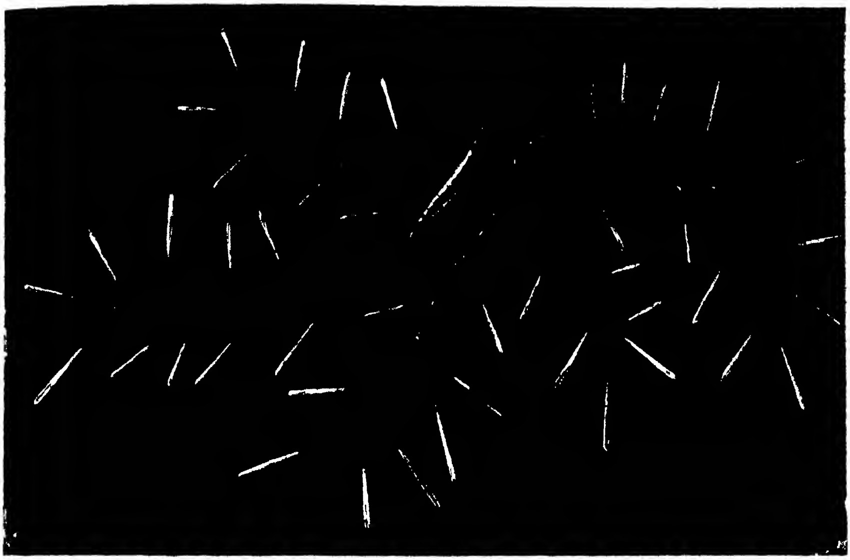
The most famous species is the New Zealand glowworm (Edwards, 6) which is found in deep ravines and caves. Its web is built on the same general plan as the above, but the pendent strands are loaded with droplets instead of a continuous coating. It has a luminous organ situated in the posterior part of the body and the light is controlled by the larva and may be turned on or off. When an insect is caught in one of the pendent strands, the larva winds up the strand and devours the insect.

NORTH CAROLINA STATE COLLEGE,
RALEIGH, N. C.

LITERATURE CITED

1. WHEELER, W. M. Demons of the dust. New York, W. W. Norton & Co. 1930.
2 MANSBRIDGE, G. H. On the biology of some Ceroplatinae and Macrocerinae. Trans. Ent. Soc. London 81: 75–92. 1933.
3. BUSTON, H. W. Note on the Chemical Nature of the fluid from the webs of larvae of Platyura and Ceroplatus. Appendix to paper by Mansbridge (2).
4. MADWAR, S. The biology and morphology of the immature stages of *Macrocera anglica*. Psyche 42: 25–34. 1935.
5. COOK, O. F. Webspinning fly larvae in Guatemalan caves. Jour. Wash. Acad. Sci. 3: 190–193. 1913.
6. EDWARDS, F. W. The New Zealand Glowworm. Proc. Linn. Soc. London 1933-34: 3–10. 1934.

PLATE 27



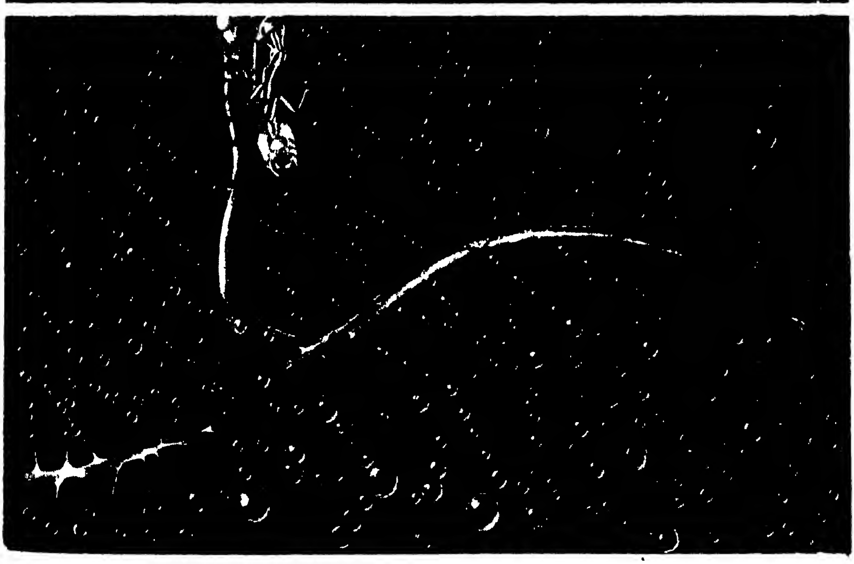


Fig. 1. (Above) Luminous larva in web. North Carolina mountains.
Fig. 2. (Below) Lochetic Mycetophilid larva from eastern North Carolina.

THE OCEAN SUN-FISHES ON THE NORTH CAROLINA COAST.

THE POINTED-TAILED *Masturus lanceolatus* AND THE ROUND-TAILED *Mola mola*

BY H. H. BRIMLEY

PLATES 28–30

On November 1, 1937, a specimen of the Long-tailed Ocean Sun-fish, *Masturus lanceolatus*, was caught by the mullet-seine crew of Brown's Inlet Fishing Camp in the sound and about half a mile south of the Inlet, Brown's Inlet being about half-way between Bogue and New River Inlets on the shore-line of Onslow County, North Carolina. The captors had placed lines around the fish's body and then anchored it in the deep water of the channel directly opposite the camp, where Harry T. Davis and I examined, measured and photographed it on the afternoon of November 2, the fish being alive at the time. It reached the Museum in the flesh about mid-day of November 8, when the body was placed on ice overnight, and the making of the plaster molds was started the morning of November 9.

The figures below show the dimensions of the specimen, with colors and other notes following:

Length, tip of snout to tip of caudal	73½"
Greatest depth of body	37"
Greatest thickness of body, partly estimated	11"
Height of dorsal fin	27"
Height of anal fin	25"
Tip to tip of dorsal and anal fins	84"
Tip of tail to proximal edge of tail muscles	19"
Pectoral fins	5¼" x 7¼"
Gill opening	2¼" x 4"
Diameter of eyes	2" x 2¼" and 2" x 2⅓"
Length of gills, 4 to each side	20"
Width of mouth when open	4"
Estimated weight	500 lbs.

Fin rays, counted from the plaster molds: P. 9; D. 18; C. (partly estimated) 18; A. 14.

Color: The sides of the body showed an uneven dirty-silvery tint, lighter below and darker above. All the fins, with the exception of the pectorals, were darker than the nearby body colors. The soft muscles at the bases of the vertical fins showed a blotchy and rather spotty effect, the general effect of these blotchy areas being somewhat lighter than the fins they supported. The upper sides and the top of the head were nearly the color of the back. A rather dark, muddy, slaty brown might be a fair description of the color of all of the fins.

When viewed alive, the color of the lips and interior of the mouth was a rather dark old rose with a trace of purple.

The mouth, when opened, was almost circular in outline, the tongue showing conspicuously. The live specimen opened and closed its mouth a number of times during our examination.

A nictitating membrane enabled the creature to open and close its eyes. I saw this happen several times and the fisherman also called attention to this feature. The color of the iris appeared to be a rather light mottled brown.

The caudal fin was so flexible at its base as to allow a side movement almost at right angles to the center line of the body, indicating the use of this organ in swimming. This is well shown in one of the photographs.

Side movements of the caudal fin were made consciously by the fish while suspended for photographing.

The anal and dorsal fins were susceptible to a considerable twisting, as well as lateral, movement.

The surface of the skin was sand-papery in feel when stroked towards the head—very much like that of most sharks—but smooth to the feel when stroked towards the tail. It was not covered with the noticeable spines and the thick, persistent slime, that are so characteristic of *Mola*.

The only hard parts found in this specimen were the bony cutting edges of the jaws that take the place of teeth. This character is much the same as in *Mola*, and the cleaned jaws bear quite a strong resemblance to those of the loggerhead sea-turtle.

An audible "grinding" of the jaws was reported by the fishermen who stated that it sounded "like a hog eating corn!" My defective hearing caused me to miss this point.

The caudal fin showed no sign of injury, being perfect in outline. Its extended portion was situated above the median line of the body, which seems to be characteristic of the species.

The specimen was in almost perfect condition, and I believe it might be regarded as quite typical in its proportions and in its coloration, except for the fact that there was a complete absence of the spots on the vertical fins that seem to be generally found on this species.

The skin adhered very closely to the underlying muscular tissue—as in *Mola*—to the point where the actual skinning of such a specimen would seem to be a rather hopeless undertaking.

The contents of the abdominal cavity were removed by Dr. Reinard Harkema, of the biological department of N. C. State College, for an examination for parasites.

Plaster casts were made of both sides of the body, including the caudal fin and the bases of both dorsal and anal fins. The pectorals, dorsal and anal were then cast separately, on both sides. A cast of the lips and the interior of the mouth was also made.

The hard jaws and the pectoral fins were preserved; also, a sample of the skin.

Dr. E. W. Gudger, Associate Curator of Fishes in the American Museum of Natural History, New York, who has made an intensive study of this species, has recently published an exhaustive and well illustrated 43-page and five-plate article on *Masturus* in the Proceedings of the Zoological Society of London, under date of September 22, 1937. In this paper, Dr. Gudger lists all known records of the species, 31 adults and a number of young in the post-larval state, and, as this fish was first described in 1840, or 1841, the small number of known occurrences of so large and conspicuous a species seems to support the published statement that it is the rarest of all large ocean fishes.

One record, however, was overlooked by Dr. Gudger, this being a photograph in a non-scientific publication of a "Sunfish," the collector evidently regarding his capture as a malformed specimen of *Mola*, though the picture identifies it as *Masturus*. The available data of this catch is as follows:

Mr. H. Wendell Endicott of Boston, Massachusetts, in his book, *Adventures with Rod and Harpoon Along the Florida Keys*, mentions the taking of a Sunfish of about 400 pounds weight in the Gulf Stream, but gives no particulars. So, as the photograph of this specimen shows it to be *Masturus*, I secured the following details from Mr. Endicott direct: It was harpooned near Alligator Light, probably off Lower Matacumba Key, in the early spring of either 1923 or 1924, Captain Walter A. Starck being with Mr. Endicott at the time. Not recognizing it as anything

out of the ordinary, no measurements were taken and no notes of the event were made other than a mere reference to the capture.

Dr. Gudger's list of 31 recorded adult specimens shows 3 from the Indian Ocean, 5 from the Pacific and 23 from the Atlantic, 9 of the latter being from the east coast of Florida. The most northern of these (2) are reported from St. Augustine, that taken in 1912 having a reported length of 10 feet and an estimated weight of 1700 pounds. And, in spite of no scientific publication of its dimensions, it would seem to be a world-beater. The mounted (stuffed) skin of this fish is on exhibition in the Museum of Marine Curiosities on Anastasia Island, St. Augustine, Florida. Dr. Gudger shows a photograph of the giant taken during the process of "stuffing."

Dr. Gudger makes the point—undoubtedly well taken—that many a *Masturus* has been mistaken for a *Mola*, particularly in cases in which the tail was not observable. And no doubt in many cases in which the caudal fin could be seen, the specimen would be classed as a *Mola* with a distorted rear-end. I fell into that error myself in first viewing the illustration in Mr. Endicott's book, not knowing of the existence of *Masturus* at the time.

On December 20, 1937, a crew of seine fishermen while heading for Masonboro Inlet, directly south of Wrightsville Beach, N. C., noted a tall fin sticking up above the level of the marsh in the sound. On approaching the object they found that a large fish of a species unknown to them had worked into a narrow slough at high tide and had become imprisoned as the tide went down.

The efforts of the whole crew of eight men were required in getting the specimen into deep water, and five men working in unison were unable to lift it. It was landed alongside the causeway leading from Wrightsville across the sound to Wrightsville Beach, where it was placed on exhibition.

A notification was received by wire that a large sunfish had been captured but, as the Museum already possessed good examples of both *Mola* and *Masturus*, no effort was made to secure this one, although there was the problem of determining the species to be considered.

The writer of this paper was fortunate enough to examine and measure the specimen on December 29, nine days after its capture, and it proved to be *Masturus*.

Mr. Burke H. Bridgers, of Wilmington, had already taken measurements, which in all cases came within an inch or two of those made by the writer. However, as mine were made after some shrinkage had

Table of Records of Masturus and Mola in North Carolina Waters

DATE	LOCALITY	SIZE	NOTES	REPORTED BY
		Masturus Lanceolatus		
May 15, 1904	Cape Lookout	"Stuffed" specimen in N. C. St. Museum. Now measures 28″ x 13″	Mentioned by Dr. Smith under *Mola*	Dr. H. M. Smith, in *Fishes of North Carolina*
Nov. 1, 1937	Brown's Inlet, Onslow County	L. 73″; depth of body, 37″; est. wt. 500 lbs.	Measured and photographed while still alive by H. T. Davis and H. H. Brimley, of the Museum. Model of this specimen in State Museum	N. C. State Museum
Dec. 20, 1937	Masonboro Inlet	L. 83″; depth of body, 45″; est. wt., 800 lbs.	Found stranded in narrow slough in marsh less than a mi. inside the Inlet	Measured by Burke H. Bridgers; observed and identified by H. H. Brimley
Dec. 22, 1937	Pamlico Sound 1 mi. west of New Inlet	"Easily 600 pounds"	The identification of this specimen is described in the text	A. C. Stratton, Manteo
		Mola mola		
1889	Cape Lookout	No dimensions given	In National Museum	Dr. H. M. Smith in *Fishes of North Carolina*
1904	Cape Lookout	L., 8′; est. wt. 1000 lbs.		Dr. H. M. Smith in *Fishes of North Carolina*
May 30, 1926	Swansboro	L. 85″, dep. 49″, est. wt., 1200 pounds	Found stranded in the sound a few miles inside Bogue Inlet. Model of spec. in State Museum	Taken by Dr. R. L. Daniels of New Bern
May 12, 1937	Oregon Inlet	Wt. 253 lbs.	No measurements taken	B. Wesley Gatch, Baltimore, Md.

sticking up above the surface of the water. His boatman, not recognizing the fin as belonging to any fish known to him, ran the boat within 75 to 100 feet of the creature and suggested to Mr. Gatch that he cast his lure—a Japanese feather jig—directly in front of the fish's mouth. The aim was true, the lure struck the water where intended and it was immediately grabbed by the fish and the hook took hold. It remained on the surface after being hooked, the boat following its movements while the angler kept his line tight. Finally, when boat and fish were not more than 20 feet apart, the hook pulled out. But, with the typical Sunfish slugginess, no effort to escape was made and the fish was finally dragged into the boat.

It was photographed and weighed, but no measurements of the specimen were made. Its weight was 253 pounds, and it may be that this is the first recorded instance of an Ocean Sunfish taking an angler's lure and being played on rod and reel. Or *was* it taken intentionally?

KEY TO SPECIES FROM EXTERNAL CHARACTERS

1. Caudal fin extending horizontally to a length of about one-fourth of the measurement from tip of snout to tip of tail, with a long lobe-like projection situated somewhat above the median line of the body. Caudal rays prominent in both fresh and dried condition, numbering 15 to 24, or more Skin denticles only noticeable when the body is stroked from the tail, forward, when a slight sand-papery effect is observed. When stroked in a backward direction the skin appears to be smooth. Vertical fins often profusely spotted. No slime on body. MASTURUS
2. Caudal not recognizable as a fin. The outer border of this thick and rigid member shows a series of rather regular scallops forming a gentle curve from the base of the dorsal to the base of the anal. Rays not distinguishable in the fresh specimen, perhaps not even in the dried tail. Dissection shows 12 or 13 rays as the usual number. Skin denticles are in the form of erect spines and are covered with a dense and persistent slime, both denticles and slime being very noticeable characteristics. MOLA

STATE MUSEUM
RALEIGH, N. C.

PLATE 28

Masturus lanceolatus from Brown's Inlet

(Above) Showing tail bent at right angle to body. Photograph by author.
(Below) Photograph by H. T. Davis.

PLATE 29

Masturus lanceolatus

(Above) From Masonboro Sound, estimated weight, 800 lbs.
(Below) Small stuffed specimen, mentioned by Dr. Smith under *Mola*, found dead at Cape Lookout, March 15, 1904.

PLATE 30

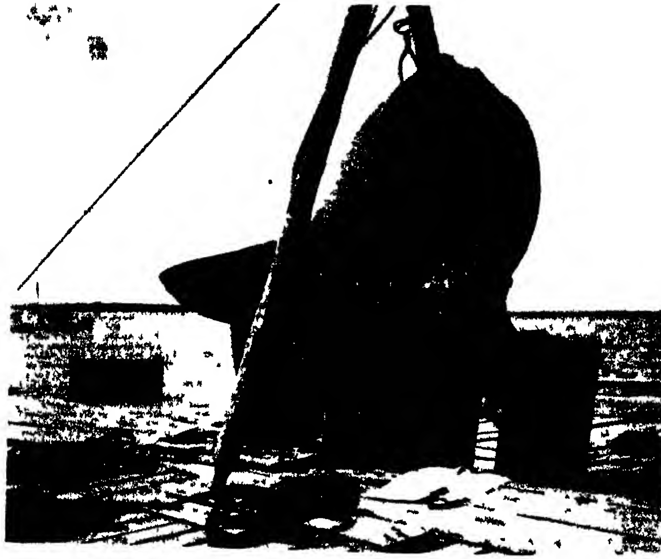
Mola mola

(Above) Specimen caught near Swansboro, N. C., May 30, 1926. Estimated weight 1200 to 1300 lbs. Photograph by J. F. Cunningham.
(Below) Specimen caught by B. Wesley Gatch of Baltimore, Md., May 12, 1937. Weight 252 lbs. Note that the projection showing below the caudal fin is the end of a rope, not a part of the tail.

PLATE 28

PLATE 29

PLATE 30



HISTORY OF THE THREE 6-INCH POST-LARVAE

On April 17, 1938, Capt. E. E. Saar in his boat "Viking" was fishing in the green-water edge of the Gulf Stream off Fort Lauderdale, Florida. Several dolphins (*Coryphaena*) were taken. When the boat landed her catch at the dock at Fort Lauderdale, the captain noticed that one of the dolphins (weighing 17 lbs.) had a very much swollen abdomen. When it was opened, the three young *Masturus* were found. The other dolphins were also opened, but unfortunately none of them contained young pointed-tails.

Every one was interested in these strange fish, but no one knew what they were. Capt. Saar carried them to Mr. Dale Redman, an agent of Mr. Pflueger, and he sent them to Miami for identification. At the first glance they were recognized, and knowing my great desire for such intermediate forms, Mr. Pflueger generously forwarded them to me for study—two of them to become the property of the American Museum. Thus through the cooperation of these three men these invaluable specimens were obtained and preserved for study.

OTHER YOUNG MASTURUS FROM THE STOMACHS OF DOLPHINS

It will interest the reader to know that these young pointed-tailed ocean sunfish are not the first to be collected from the stomach of a *Coryphaena*—even in Florida waters. The earliest specimens of *Masturus* ever recorded from the waters of this state were four little fellows taken from the stomach of a dolphin captured off Pensacola in 1882. These measured (to base of caudal fin—the only measurements given) 35, 39, 42, and 50 mm. The smallest of these is shown herein in natural size as Fig. 1. It is the only small spotted specimen heretofore figured and described. Its "over all" measurement is 49 mm.—the filament giving the added 14 mm.

Still earlier (1880) the "Blake" expedition captured a dolphin in the western Sargasso Sea (Lat. 31° 30′ W. and Long. 73° 31′ W.) east of Savannah, Ga. From its stomach were taken two small *Masturus*. These remained unidentified and undescribed in the Museum of Comparative Zoology, Cambridge, Mass., until I figured and described one of them in 1935. It measured 53 mm. in standard and 65 mm. in over all length. It is portrayed in Figure 2.

There is in the Copenhagen Museum a little *Masturus* taken from the stomach of a *Coryphaena* captured off the Azores Islands (distance and date not given). This small specimen (shown in Fig. 3 herein) measured

47.5 mm. in standard and 54 mm. in over all length. The difference is due to the long central "whisker" of the caudal.

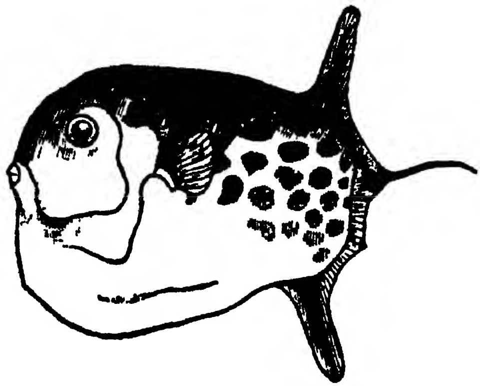


FIG. 1. A 59 mm post-larval *Masturus*, with spots and whiplash tail, taken from the stomach of a dolphin caught off Pensacola, Florida. After Perugia, 1889.

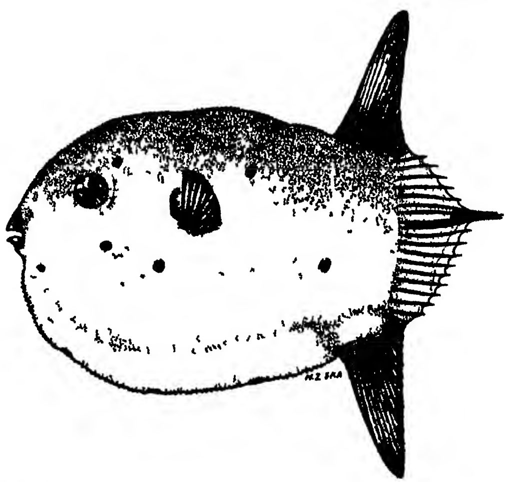


FIG. 2. A post-larval pointed-tailed ocean sunfish, 65 mm in total length, taken from the stomach of a dolphin captured in the western Sargasso Sea. After Gudger, 1935

The young post-larval pointed tails thus far noted were all taken from the stomachs of dolphins caught in the North Atlantic—the 3 from off Ft. Lauderdale, 4 from the Gulf of Mexico off Pensacola, and 3 from various parts of the open ocean.. But the Central Pacific has also con-

tributed 3 larvae. These were taken in 1911 on a traverse from the Ellice Islands to the Union Group, latitude and longitude not given.

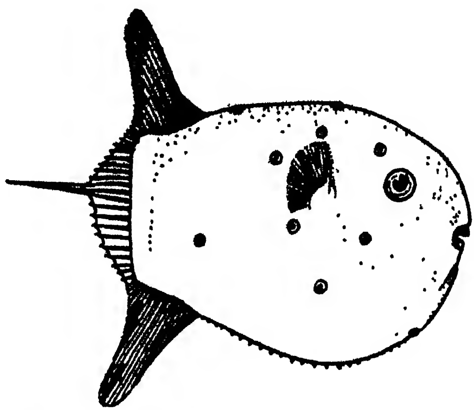


FIG. 3. A post-larval *Masturus* with whiplash tail (54 mm. in total length) taken from the stomach of a dolphin hooked off the Azores Islands. Redrawn from Steenstrup and Lütken, 1898.

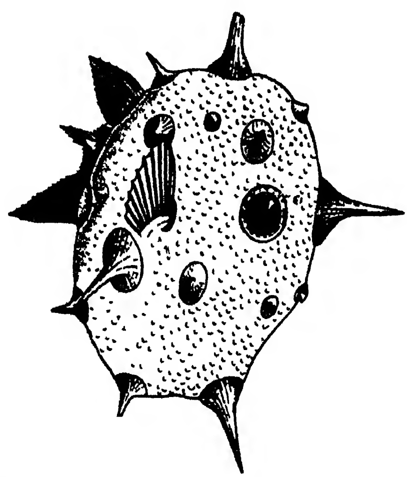


FIG. 4. A larval *Masturus* (10 mm. long) taken from the stomach of a kingfish captured in the Central Pacific Ocean. Note the spines and the curious tail-structures. After McCulloch, 1912.

They were mere "babies," 9.5, 10 and 13 mm. long, and, as Fig. 4 shows, they still possessed the spines and the curious tail structures, so charac-

teristic of the larval forms. These, however, came not from the stomach of a dolphin, but from that of another predatory fish—a kingfish.

From the above, it will be seen that of the 28 larval and post-larval forms which I was able to list in 1937, 10 (more than one third) came from the stomachs of predatory fish. To these must now be added Mr. Pflueger's three from off Fort Lauderdale, Florida, making a total of 13 out of 31 described young. In the light of this evidence, it is to be hoped that readers of this article who are deep-sea anglers will examine in their catches the stomachs of dolphins, kingfish, and like large predators, in the hope that they may find other specimens of the young *Masturus*.

THE SIX-INCH SPECIMENS DESCRIBED

These specimens are practically all of a size in standard length; 125, 127, 130 mm. In total length they measure 152, 157, and 160 mm. The differences in total lengths are due to the varying lengths of the caudal lobes. Since these specimens are so nearly of a size, it seems not improbable that they were from a school of one hatching. The smallest is in perfect condition, but a larger specimen shows marked evidence of considerable action of the digestive juices of the dolphin on its right hinder parts. On both sides the abdominal wall is noticeably discolored, the left side being very dark. The largest fish has a very much discolored wall on the left side. On the right side there is in the abdominal wall a tear about 30 mm. long, extending diagonally in a line joining the base of the pectoral and the front edge of the base of the anal fin. Through this some of the viscera protruded. This tear seems to be a mechanical injury. There does not seem to be any food in the alimentary canal of any one of these late post-larvae.

To get the facts as to sizes, fin-ray counts, etc., briefly before the reader, they are set out in the following table.

NO.	LENGTH			DEPTH			FIN-RAY COUNT				D + C + A COMBINATION
	Standard	To Edge C	To Tip C	Behind Eye	Before D & A	Over D & A	P	D	C	A	
1	125 mm. 4.9 in.	147 mm. 5.7 in.	152 mm. 6 in.	67 mm. 2.6 in.	72 mm. 2.8 in.	145 mm. 5.7 in.	10	19	6 + 6 + 9	18	58
2	127 mm. 5 in.	148 mm. 5.75 in.	160 mm. 6.3 in.	68 mm. 2.65 in.	73 mm. 2.9 in.	145 mm. 5.7 in.	10	20	7 + 6 + 12	17	62
3	130 mm. 5.1 in.	150 mm. 5.9 in.	157 mm. 6.2 in.	67 mm. 2.6 in.	72 mm. 2.8 in.	141 mm. 5.6 in.	10	19	7 + 5 + 11	18	60

A comparison of total lengths with depths before dorsal and anal fins shows that these fish are short—but deep-bodied. And especially when one compares the length of the body proper (standard length) with the depth over the unpaired fins, one finds a justification for the family name, Molidae—Latin *mola*, a mill-stone. However, the genus *Mola* has a shorter and more rounded outline than *Masturus*, while on the other hand, *Ranzania*, the third genus, with its tail cut obliquely, is still longer-bodied than *Masturus* in proportion to the depth, taken both before and over the dorsal and anal fins.

A further careful study of this table shows what a closely-graded series these three little fish form. The variations in measurements are somewhat affected by the degree of digestion, which each fish had suffered, and by the differential shrinking of each fish as it was preserved in alcohol. One has to have the three fish laid out side by side to see how nearly identical they are. Fish No. 1 is shown in Figure 5—an accurate portrayal. In each fishlet the jaws are well developed, and the teeth are sharp and fully functional for feeding. The eye and the gill orifice with its single flap are entirely like those of the adult. The skin is everywhere rough to the touch. This is especially true of the dorsal ridge, while the sharp edge of the venter is beset with prickles pointing slightly backward. The spines which are so characteristic of the early larval stages (Fig. 4) are gone, but on each fish I find one or more horny bases left by the spines when they fell off, as are shown in Figs. 2 and 3.

All the little fish are wedge-shaped, thicker on the dorsal surface and tapering almost to a dull-knife edge on the serrate venter. As may be seen in Figs. 1 and 2, below the abdominal cavity there is a thin transparent region, apparently made of skin only and forming a kind of keel with a serrate edge. This was first noted in 1871 by F. W. Putnam of specimens from Massachusetts Bay. The dorsum is broadest over and just back of the eye, and thins out toward the region of the dorsal fin as well as towards the venter. The fish in alcohol are dark on the dorsum and a dirty light-brown on the sides. Dorsal and anal fins are also dark. There is some evidence that the left side of the anal fin of fish no. 1 was spotted. But the hinder part of the left side of the body and caudal fin also show fair reminiscences of spotting such as was found on another and smaller specimen (Fig. 1) taken from the stomach of another dolphin. Fig. 5, portrays fish No. 1 from the left side—the conventional pose. To this has been transferred the remnants of the spotting from the right side of fish No. 2—the one with the 30-mm. tear in the left

abdominal wall. Presumably this fish (and likely the others also) possessed this characteristic coloration before it was removed by the digestive juices of the predator. The adult fish (presented to the Museum by Mr. Pflueger) which came fresh in ice, was spotted over the sides (especially the dorsal parts), over the tail-region, and on the base of the anal fin. I am told by those who have fortunately been in at the

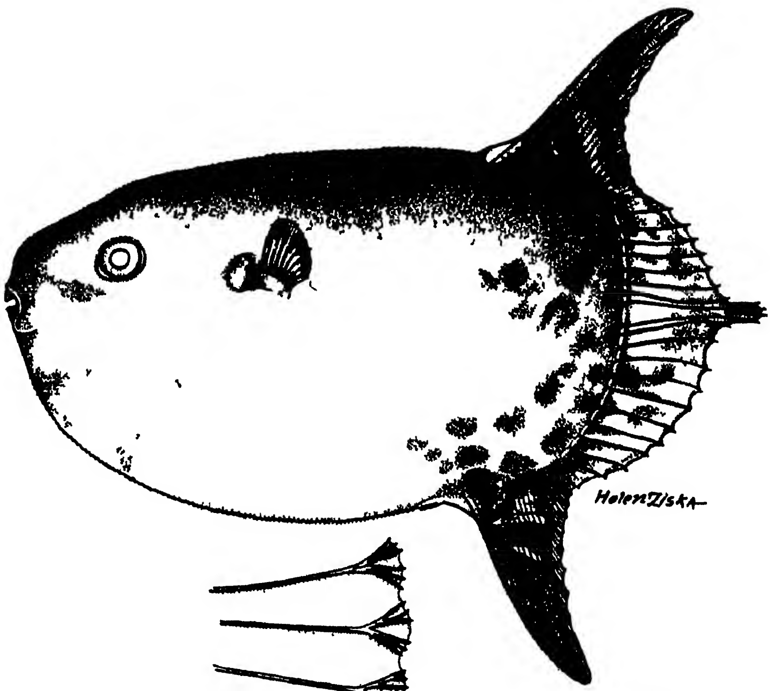


FIG. 5 A late post-larval *Masturus* (152 mm , 6 in in total length) from the stomach of a dolphin caught off Fort Lauderdale, Florida, in 1938 The inset drawing shows the much enlarged tips of the caudal fin rays.

capture of *Masturus*, that the live fish is beautifully spotted, but that the colors are apt to disappear shortly after death.

The body has no tail-region such as is found in other fishes, even near relatives of the Molidae. The body ends abruptly and the caudal fin begins as abruptly—the separation being marked in these post-larvae as in the adult by a curious band. At this band the spines of the dorsal fin are hinged. Outside and behind this band is a transparent section

of the caudal fin about 5–7 mm. wide composed of a thick rubber-like material. The remaining (outer) part of the caudal fin, including the central lobe, is paper thin. When a bleached-out fin is held to the light, this is very apparent. The fish with the spotted tail-fin shows this imperfectly, and the fin seems unusually thick—possibly because of little digestive action on it. This thinness of caudal fins in these fish emphasizes that these young are still in the post-larval stage. The caudal of the adult is covered with thick rough skin and the rays are embedded in a solid layer of collagen, as dense as rubber. When the transition is made in the structure of the fin is a problem to be solved by a study of the older post-larval forms approaching young adulthood. Notable in both post-larvae and adults is the fact that the point of the caudal is always above the median line of the fish's body.

Of particular interest are the fins and especially those which make up the dorsal-caudal-anal complex. The pectoral-fin count (10) is easy to make and in these three young fish is identical. It is difficult to make the other counts because it is hard to delimit dorsal from caudal, and to say where caudal leaves off and anal begins. In these particular specimens, counts of dorsal and anal spines are, however, made somewhat easier because the digestive juices of the dolphin have removed much of the epidermis and with it the color over the dorsal and anal fins and perhaps only to a lesser extent over the caudal—and particularly over its point.

In the first fish (Figure 5), the caudal has 6 rays above the point, 6 in this and 9 below. The caudal thus has 21 rays—of which 2 are the fin rays with invisible bases in the center of the "point." Dorsal and anal ray counts are normal, as is the count for the D + C + A complex. For fish no. 2 the caudal counts are 7 + 6 + 12 = 25. The lobe has the 4 main rays plus 2 intermediate ones which break up brush-like at the tip and make the count difficult. The total D + C + A combination = 62 is not beyond the limits of variation. Fish no. 3 had the epidermis gone off the bases of D. and A., and their counts were not too difficult. But the caudal had some color on the left side and a larger amount on the right. This caused trouble, but the counts for the upper and lower portions may be taken as they stand. The tip has much color on both sides and hence the count is difficult. I can make out the upper and lower pairs of rays as shown in Fig. 5. In between these there is certainly one supernumerary ray. It may be that there is another or that the one, which is surely there, breaks up into a brush of points.

Another curious and interesting thing, discovered by my artist and

shown in inset drawing, is the di- and trichotomous branching at the tips of the caudal rays. When digestion has not gone too far, these curious tips of the fin-rays are found everywhere, but even with digestion they are apt to persist at the ends of the rays at the junction of the caudal with the dorsal and anal fins. Furthermore, there is evidence that the hinder and outermost of the dorsal and anal rays possess such tips. These curious little structures have never before been figured, nor referred to, so far as I know.

The fortunate find of these three six-inch specimens makes possible the first step in bridging the gap between the largest post-larva (2.75 in.) heretofore known and the smallest adult—a specimen 37.5 in. over all. What is greatly hoped for is a series of forms measuring about 12, 18, and 25 in. long, in which to study the development of the post-larval tail into that of the young adult.

HONORARY ASSOCIATE
AMERICAN MUSEUM OF NATURAL HISTORY
NEW YORK, N. Y.

LITERATURE CITED

GUDGER, E. W. 1937. 1. The structure and development of the pointed-tail of the ocean sunfish, *Masturus lanceolatus*. Ann. Mag. Nat. Hist. 10th ser., 19: 1–46, 2 pls., 21 text-figs.

——— 1937. 2. The natural history and geographical distribution of the pointed-tailed ocean sunfish (*Masturus lanceolatus*) with notes on the shape of the tail. Proc. Zool. Soc. London, ser. A, pt. 3, 353–396, 5 pls., 24 text-figs.

PUTNAM, F. W. 1871. On the young of *Orthagoriscus mola* [*Masturus lanceolatus*] Proc. Amer. Assoc. Adv. Sci. for 1870, 19th Meet., 255–260, 4 figs. Amer. Nat. 4: 629–633, 4 figs.

RAVEN, HENRY C. 1939. On the anatomy and evolution of the locomotor apparatus of the nipple-tailed ocean sunfish (*Masturus lanceolatus*). Bull. Amer. Mus. Nat. Hist. 76: 143–150, text-fig., folding plate.

EMBRYONIC GROWTH RATE AND SEX DIFFERENTIATION

By Bert Cunningham

The studies of Riddle on the metabolic rate of dove eggs in relation to the sex of the developing embryo needs to be extended through the examination of other animals. To that end the following experiments were undertaken, hen eggs being the experimental material.

Pure single-comb white Leghorn eggs were secured in part from L. G. Cheek, a local poultry man, and the remainder from the Champion Poultry Farm, near Mt. Airy. These were incubated in some experiments for 9 days, in others for 11 days and still others for 14 days. It was much more difficult to be certain of the sex of the embryo at the shorter period of incubation, but such errors were offset by the higher death rate at the longer period, especially in those eggs incubated under increased atmospheric pressure. The sex of each embryo was determined by histological examination.

It has already been shown that increased pressure accelerates to a considerable degree the rate of development during the first 10 days of incubation (Cunningham, 1936). Whether this is associated with a higher metabolic rate may be debatable, but in this paper it is assumed that increased growth rate is the result of increased metabolic rate. If the metabolic rate is in any way responsible for the determination of sex then the increased rate of metabolism, as indicated by growth, should cause some shifting of the sex ratio. If the increased metabolism is rather the result of the difference of metabolic rate between the male and female soma, there should be no aberration of the normal sex ratio.

In experiments dealing with the shifting of the sex it would be highly desirable to find a species in which it would be possible to predict the "potential" sex of an egg before it is placed under experimental conditions.

Riddle (1916) reported such a condition to exist in his doves. Dove eggs, however, are difficult to secure in sufficient quantities unless a very large colony is maintained. Pigeon eggs, which are more easily obtained, and which also show size difference in a clutch, were examined; but it was found in the eggs available that neither the size nor the first or second egg of the clutch could be used as a criterion. The details of these studies on the pigeon egg will be reported later.

Hen eggs were examined to see if any correlation existed between the weight of the egg and the sex of the embryo. The data will be presented later, but suffice it to say here no relationship was to be found.

It has been reported that a given bird lays a higher percentage of potential male eggs in its earlier laying and that as the season progresses the relative number of potential females increases and later exceeds the males. For that reason no general control may be set up; a control series consisting of eggs taken at the same time as the experimentals must be set up for each experiment.

If there is a differential-sex metabolism one might expect as a result an increase in the growth rate of the embryo, with the result that embryos of one sex would be larger than those of the other sex at comparable incubation times. That such differences do not occur in these experiments should be stated here although the data will be presented later.

From such preliminary studies as those noted above it is evident that the sex of a hen egg can not be predicted and while the ideal conditions for an experiment would involve the accurate pre-determination of the normal sex of an egg, and an effort to produce an embryo of the opposite sex from this egg, that appears impossible under our present circumstances. The method of approach used herein, therefore, is open to criticism, and the results may not be considered as conclusive.

Since one cannot predict the sex of the embryo by the size of the hen egg or embryo, one has to assume that the sex ratio in the experimental eggs will approximate that found in the control eggs. The comparatively small number of eggs involved in each experiment may in a small degree invalidate such an assumption, and to be significant any change in sex-ratio in experimental eggs would have to be considerably divergent from the control sex-ratio. However, such divergence as there is extends in both directions in these experiments and thus lessens the weight of the objection.

It might also be argued that pressure incubation produces a differential death rate. While the data show ratios comparable to the normal, in which it has been shown there is no differential death rate, it is not impossible that some of the potential females are shifted to compensate for a higher death rate of the males. There seems to be no method for settling this point at present.

The work is further open to criticism since in several experiments there is a heavy egg loss due to early death of the embryos. However, one experiment in which 90 per cent of the eggs resulted in embryos in which the sex could be determined seems to invalidate this objection.

The procedure in these experiments has been to match, by weight, the experimental and control eggs and after incubation to use the sex ratio of the control eggs as a basis for determining whether or not the enforced growth rate has any effect upon the sex ratio of the experimental eggs as compared to the controls. Studies were made, on both control and experimental, to determine whether there was any relation between the original weight of the egg and the sex of the embryo, or between the weight and sex of the embryo. Studies were not made on yolk size and sex but they will be undertaken as soon as eggs become available again.

Five separate experiments, involving 367 embryos, the sex of 345 of which was determined, were conducted.

TABLE I

Distribution of Male and Female Embryos in Relation to Egg Weight

Normal Pressure

Egg Weight		56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	Grams Total
No. of Embryos	24	4	4	7	6	8	5	5	4	4	4	5	4	3	1	2		90 ♂
	13	5	4	4	6	5	8	6	6	8	4	7	1	2	2		1	82 ♀

TABLE II

Original Egg Weight and Sex

Pressure Incubation

Egg Weight	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	73	75	Grams Total
No. of Embryos	4	2	5	9	5	7	6	5	12	8	5	1	7	5	5	2		1	95 ♂
	2	4	6	3	11	3	9	7	8	12	6	4	4	5	4	2			98 ♀

In his doves, Riddle (1930) found the first laid egg of a clutch to be smaller than the second, and it usually developed into a male; the second developed usually into a female. Such a condition might suggest that the smaller hen eggs might produce an excess of males and the larger ones an excess of females. While it was not feasible to collect eggs from a single hen, eggs taken at random from a large poultry farm should give a fair sample. When such eggs are weighed, placed under normal incubation and the sex of the embryo determined histologically there is no evident relation between the weight of the egg and the sex of the embryo, except in the extremely small eggs. (See Table I.)

Note: In the preparation of Tables I and II, perpendicular lines have

been drawn through unit weights. The number of embryos represent those that fall between the weight indicated at the left and under the weight designated at the right. For example, the seven male embryos of Table I weighed 58 grams or more, but less than 59. Those to the left of 56 grams, weighed less than this, but were too widely distributed to be further separated.

When the sex of "pressure" embryos is studied in relation to the original weight of the egg the distribution appears to be comparable to that of the normal development. Table II should be compared with Table I.

TABLE III

Average Weights of Male and Female Embryos at Various Stages of Normal Incubation

	9 DAYS	11 DAYS	14 DAYS
Av. Wt. of eggs	58.28	58.9	65.2
Av. Wt. of embryos ♂	2.33	3.60	11.5
Av. Wt. of embryos ♀	2.355	3.81	10.81
Av. Wt. of embryos ♂ & ♀	2.34	3.68	11.3

TABLE IV

Average Weights of Male and Female Embryos at Various Stages of Pressure Incubation

	9 DAYS	11 DAYS	14 DAYS
Av. Wt. of eggs	58.6	59.7	66.2
Av. Wt. of embryos ♂	3.00	6.084	12.4
Av. Wt. of embryos ♀	3.06	6.055	12.3
Av. Wt. of embryos ♂ & ♀	3.04	6.07	12.34

Although the growth rate of these embryos has been accelerated by more than 40 per cent during the period of sexual differentiation there is no evidence that the potential sex has been overridden in any significant manner by the increased metabolism.

Studies on the relation of the weight of embryos to sex were also made. Under normal incubation the differences in weights of male and female embryos do not appear to be significant. (See Table III.)

While there is considerable increase in the growth rate of the embryos under pressure, the differential between the males and females is not

significantly different, being well within the limits of probable experimental error. (See Table IV.)

Since the season in which the eggs were laid might have some bearing on the sex ratio, several experiments have been brought together in one table. (See Table V.)

When the ratios of pressure and controls are examined there is comparatively little difference, certainly under the conditions of the experiment this is not significant.

Preliminary studies on pigeon eggs indicate that similar results are to be expected.

From these data it is quite evident that no material change has been made in the sex ratio by the increased atmospheric pressure.

TABLE V

DATE SET	CONDITION	NO. OF EGGS	INCUBATION PERIOD	NO. OF EMBRYOS	MALE	FEMALE	UNDETERMINED
Feb. 28	Control	38	14 days	33	17	16	None
Feb. 28	Pressure	58	14 days	25	14	11	None
Apr. 1	Control	56	14 days	50	21	29	None
Apr. 1	Pressure	58	14 days	48	20	28	None
Apr. 7	Control	40	9 days	38	21	16	1
Apr. 7	Pressure	43	9 days	34	17	17	None
Apr. 13	Control	64	9 days	38	14	14	10
Apr. 13	Pressure	54	9 days	38	15	15	8
Apr. 22	Control	32	11 days	32	19	13	None
Apr. 22	Pressure	40	11 days	31	14	14	3
Total		483		367	172	173	22

If the size of the embryo is any indication of the metabolic rate, then sex differentiation cannot be attributed to metabolic rate since there is no correlation between the size of the embryo and its sex either in control or experimental animals.

If we assume that the increased growth rate brought about by increased atmospheric pressure is accompanied by a higher metabolic rate, then it must be concluded that there is no relationship between the metabolic rate and sex determination in the single-comb white Leghorn fowl.

While these results represent negative evidence their publication seems justified since Riddle (1930) says, "Wherever a relation between metabolism and sex has been adequately sought, it has been found—

and the same type of metabolic difference everywhere coincides with one and the same type of sex difference."

DEPARTMENT OF ZOOLOGY,
DUKE UNIVERSITY,
DURHAM, N. C.

LITERATURE CITED

CUNNINGHAM, B. 1936. The incubation of hen eggs under increased atmospheric pressure. Journ. Elisha Mitchell Sci. Soc. **52**: 269–273.

RIDDLE, O. 1916. Sex control and known correlations in Pigeons. Am. Nat. **50**: 385–410.

RIDDLE, O. 1930. Metabolism and Sex. Proc. 2nd. Internat. Congress for Sex Research, Vol. **2**: 180–189.

EXTENDED RANGES OF SOME AQUATIC INSECTS IN NORTH CAROLINA[1]

By Thelma Howell

During the summers of 1938 and 1939 the writer collected larval and adult forms of aquatic insects from ninety-seven stations located in Macon, Jackson, Swain, Haywood and Transylvania counties. When the species obtained were listed and compared with the records from North Carolina as reported by Brimley (1938), new records for the state were noted. It is the purpose of this paper to report those species new to the state and to indicate new ranges and seasonal records for others. Specimens of the species mentioned are deposited at Duke University unless otherwise noted.

Dr. A. S. Pearse is responsible for the writer's interest in the aquatic fauna of rapidly flowing streams. In a large measure, his aid and criticisms have made possible the work reported here. Grateful acknowledgment is also made of the help and many courtesies extended the writer by President W. C. Coker of the Highlands Museum and Biological Laboratory. The writer wishes to acknowledge the help which the following specialists have given in the identification of specimens: Odonata, J. G. Needham; Tricoptera, H. H. Ross; Plecoptera, T. H. Frison.

STATIONS STUDIED

The stations worked included small spring brooks, but for the most part they were located on the following larger bodies of water:

Macon County: Ball Creek, Beasley Creek, Big Creek, Buck Creek, Buckeye Branch, Burning Town Creek, Caler Fork Creek, Choga Creek, Clear Creek, Cold Spring Creek, Cullasaja River, Dirty John Creek, Edwards Creek, Jarrett Creek, Kline's Lake, Lake Randall, Lake Ravenel, Lake Sequoyah, Matlock Creek, Nantahala River, Otter Creek, Overflow

[1] The work reported here was done while holding the Duke University scholarship at the Sam T. Weyman Memorial Laboratory, Highlands, N. C., in 1938, and while using the Duke University space there in 1939.

Creek, Shope Creek, Skittles Creek, Tessentee Creek, Wayah Creek, White Oak Creek, Wine Spring Creek.

Jackson County: Chattooga River, Cullowhee Creek, Fowler Creek, Green's Creek, High Hampton Lake, Knob Creek, Lake Sapphire, Norton Mill Creek, White Rock Creek, Slicken

TABLE 1

Physical Factors in Waters Near Highlands, N. C.

STATIONS AND DATES	TIME	TEMP. °C.		pH	O_2 CC. PER LITER
		Air	Water		
May 24, 1939					
Small pond, Highlands, N. C.	12:25 P.M.	24.5	15.5	5.8	4.21
May 26, 1939					
Large stream, Primeval Forest			17	6.2	5.89
June 6, 1939					
Spring brook, Highlands, N. C.		24	16	5.2	
June 13, 1939					
East Fork Tuckaseegee River		25.5	19	6.8	6.23
June 20, 1939					
Lake Randall	12:45 P.M.	25	23	6.5	5.22
Big Creek, above Lake Randall	2:00 P.M.	25	19	7.0	5.76
June 22, 1939					
Cullasaja River at Gneiss		31	21.5		
Cullasaja River at Gold Mine Cr.		24.5	22.5		
July 10, 1939					
Bradley Fork, Great Smoky Mt. Nat. Park	2:15 P.M.	26	17	6.8	
July 11, 1939					
Deep Cr., Great Smoky Mt. Nat. Park	11:00 P.M.	31	19.5	7.4	
July 12, 1939					
High Hampton Lake, Cashiers, N. C.	10:45 A.M.	25.5	23.5	6.4	4.93
	1:40 P.M.	29	26		5.20
	3:50 P.M.	30	26		5.24
	5:00 P.M.	29.5	26.5		5.54
July 20, 1939					
W. Fork Tuckaseegee River	11:45 A.M.	27.5	19.5	7.2	5.64

Creek, Soco Creek, Tennessee Creek, Tuckaseegee River (East and West Forks).

Swain County: Bradley Fork, Deep Creek, Forney Creek, Hazel Creek, Indian Creek, Oconalufty River.

Transylvania County: Toxaway Falls and Toxaway River.

PHYSICAL FACTORS

Surface temperatures of the waters ranged from 15°C–26°C, pH 5.8–7.4, and oxygen in cubic centimeters per liter 3.58–6.30. Samples of the data obtained are shown in Table 1.

ORDER ODONATA, The Dragon-flies
Sub-order ANISOPTERA, The Dragon-flies proper
Family AESCHNIDAE
Sub-family PETALURINAE

Tachopteryx thoreyi Hagen
Macon County: Aug. 11, 1939, adult, Ammons Picnic Ground, Nantahala National Forest.
Remarks: Previously reported only from Wake and Wilkes counties (Brimley, 1938).

Sub-family GOMPHINAE

Lanthus albistylus Hagen
Macon County: May 30, 1939, nymphs, Junction Ball and Shope Creeks.
Jackson County: 1939–June 13, nymphs, West Fork Tuckaseegee River; June 16, adult female, West Fork Tuckaseegee River; July 6, nymphs, Tennessee Creek; July 12, nymphs, tributary of High Hampton Lake.
Remarks: Additional records for nymphs and specific locality for adult recorded.

Family LIBELLULIDAE
Sub-family CORDULINAE

Neurocordulia sp.?
Macon County: May 21, 1939, nymph, Lake Ravenel, Highlands.
Jackson County: June 28, 1939, nymph, Norton Mill Creek.
Remarks: Genus recorded for state only once (Brimley, 1938).

Tetragoneuria cynosura Say
Macon County: May 29, 1939, adult, Lake Ravenel, Highlands.
Jackson County: June 2, 1939, adult, High Hampton Lake, Cashiers.
Remarks: Range greatly extended and new seasonal record for mountain region given.

Sub-family LIBELLULINAE

Libellula auripennis Burm.
Macon County: July 1939, adult, Highlands.
Remarks: Previous record for westward limit Raleigh (Brimley, 1938).

Libellula cyanea Fab.
Jackson County: July 12, 1939, adult, High Hampton Lake, Cashiers.

Remarks: Previous record for westward limit, Marion (Brimley, 1938). Specimen deposited in Cornell University Collection.

Libellula incesta Hagen

Jackson County: July 12, 1939, adult, Lake High Hampton, Cashiers.

Macon County: July 24, 1939, adult, Lake Ravenel, Highlands.

Remarks: Westward range extended from Raleigh (Brimley, 1938) to Highlands.

Erythrodiplax minuscula Rambur

Macon County: July 1939, adult, Highlands.

Remarks: Previous record for westward limit, Marion (Brimley, 1938).

Sub-order ZYGOPTERA, The Damsel-flies
Family COENAGRIONIDAE

Enallagma hageni Walsh

Jackson County: June 2, 1939, adult, High Hampton Lake, Cashiers.

Remarks: Only other record for state from Highlands (Brimley, 1938).

Amphiagrion saucium Burm.

Macon County: May 29, 1939, adult, Lake Ravenel, Highlands.

Jackson County: June 8, 1939, adult, Lake Sapphire, Cashiers.

Remarks: Additional records for mountain region.

Chromagrion conditum Hagen

Macon County: May 20, 1939, adult, Lake Ravenel, Highlands.

Remarks: Extension of seasonal range for Highlands from June to May.

Ischnura verticalis Say

Macon County: May 24, 1939, nymph and adult, Lake Sequoyah, Highlands.

Remarks: Extension of seasonal range for Highlands area from July to May.

ORDER PLECOPTERA, The Stone-flies
Family PTERONARCIDAE

Pteronarcys proteus Newman

Macon County: 1938—June 3 and Aug. 24, nymphs, Big Creek; Aug. 16, nymphs, Wayah Creek; Aug. 26, nymphs, Edwards Creek. 1939—May 30, nymphs, Junction Ball and Shope Creeks; June 20, nymphs, Big Creek.

Jackson County: 1938—Aug. 10, nymphs, Norton Mill Creek; Aug. 11, nymphs, Cullowhee Creek; Aug. 19, nymphs, Fowler Creek; Aug. 28, nymphs, Chattooga River. 1939—May 29, adult male, headwaters Whitewater River; June 13, nymph,

East Fork Tuckaseegee River; June 14, nymph, Slicken Creek; June 18, nymph, Tuckaseegee River.

Remarks: New record for state. Adult male, headwaters Whitewater River, deposited in Illinois Natural History Survey Collection.

Family PERLIDAE

Perla hastata (Banks)

Macon County: Aug. 26, 1938, nymphs, Edwards Creek.

Jackson County: Aug. 28, 1938, nymphs, Chattooga River. 1939—March 20, nymphs, Fowler Creek; March 21, nymphs, Norton Mill Creek; June 2, nymphs, waterfall on U. S. Highway #64.

Remarks: Additional records for state.

Perla bilobata Needham and Claassen

Macon County: 1939—May 30, nymph, Nantahala River; June 30, nymph, Big Creek.

Remarks: New range. Only other record, Black Mountain (Brimley, 1938).

Neophasganophora capitata (Pictet)

Macon County: May 30, 1939, nymph, Nantahala River.

Jackson County: June 13, 1939, nymph, East Fork Tuckaseegee River.

Remarks: Specific locations in North Carolina not given by Brimley (1938) nor by Needham and Claassen (1925).

Togoperla immarginata (Say)

Macon County: 1938—June 3 and Aug. 24, nymph, Big Creek; Aug. 15, nymphs, Wayah Creek. 1939—May 30, nymphs, Junction Ball and Shope Creeks; June 22, nymphs, Cullasaja River; June 24, nymphs, Lower Cullasaja River Gorge.

Jackson County: 1938—Aug. 11, nymphs, Cullowhee Creek; Aug. 21, nymphs, Soco Creek; Aug. 28, nymphs, Chattooga River. 1939—June 18, nymphs, Tuckaseegee River.

Transylvania County: June 4, 1939, nymphs, Toxaway Falls and Toxaway River.

Remarks: Westward range greatly extended.

Alloperla lateralis Banks

Macon County: May 24, 1939, adult female, Highlands.

Remarks: Only previously reported from type locality, Black Mountain (Brimley, 1938).

Neoperla clymene (Newman)

Macon County: June 24, 1939, nymphs, Lower Cullasaja River Gorge.

Remarks: Previous record for state, Black Mountain (Brimley, 1938).

Perlesta placida (Hagen)

Macon County: 1939—June 22, nymphs, Cullasaja River, Skittles Creek; June 24, nymphs, Lower Cullasaja River Gorge.

Jackson County: 1938—Aug. 3, 10, 14, 19, nymphs, Norton Mill Creek; Aug. 11, nymphs, Cullowhee Creek; Aug. 19, nymphs, Fowler Creek.

Transylvania County: June 4, 1939, nymphs, Toxaway Falls.

Remarks: Not previously reported west of Southern Pines (Brimley, 1938).

Family NEMOURIDAE

Nemoura venosa Banks

Haywood County: June 18, 1939, adult male, small stream behind Morrison Fish Hatchery.

Remarks: Previously reported from Raleigh (Brimley, 1938).

Nemoura wui Claassen

Macon County: May 26, 1939, adult male, adult female, two nymphs, stream in Primeval Forest, Highlands.

Remarks: New record for state.

Leuctra carolinensis Claassen

Haywood County: June 18, 1939, adult male, small stream behind Morrison Fish Hatchery.

Remarks: Previously reported from type locality, Black Mountain (Brimley, 1938). Specimen deposited in Illinois Natural History Survey Collection.

Leuctra biloba Claassen

Jackson County: June 4, 1939, adult male, Norton Mill Creek.

Remarks: New record for state. Specimen deposited in Illinois Natural History Survey Collection.

ORDER TRICOPTERA, The Caddis flies
Family RHYACOPHILIDAE

Rhyacophila fuscula Walker

Macon County: 1938—June 3, larvae and adult female, Big Creek; Aug. 24, pupae, Big Creek; Aug. 25, pupa, Overflow Creek. 1939—May 30, larvae, Junction Ball and Shope Creeks, Jarrett Creek, Nantahala River; June 20, larvae and pupa, Big Creek; June 27, larvae, Cold Spring, Otter and Choga Creeks; July 5, larvae, Tessentee Creek; July 26, larvae and pupae, Beasley and Matlock Creeks; July 27, adult male, Highlands Falls; July 28, pupae, Big Buck Creek.

Jackson County: 1939—June 2, larvae, waterfall on U. S. Highway #64; June 13, larvae, East Fork Tuckaseegee River; July 6, pupa, Tennessee Creek.

Swain County (Great Smoky Mountains National Park) 1939—July 2, larvae, Oconaluftу River; July 11, larvae, Deep Creek; Aug. 13, pupa, Forney Creek.

Remarks: Range extended to Macon and Jackson counties.

Rhyacophila nigrita Banks

Macon County: May 29, 1938, pupa, tributary of Cullasaja River.

Remarks: Specimen deposited in Illinois Natural History Survey Collection. Only type locality, Black Mountain, previously reported (Brimley, 1938).

Mystrophora nigrior (Banks)

Macon County: 1939—May 30, pupae, Nantahala River; June 22, larvae and pupae, Cullasaja River; June 24, larvae and pupae, Lower Cullasaja River; June 27, larvae, Wine Spring Creek; June 27, larvae and pupae, Otter and Choga Creeks; July 5, larva, Buckeye Branch; July 26, larvae, Caler Fork, Beasley and Matlock Creeks; July 28, larvae, Big Buck Creek.

Jackson County: 1939—June 13, pupae, East Fork Tuckaseegee River; June 14, pupae, Slicken Creek; June 18, larvae and pupae, Tuckaseegee River; July 6, larvae and pupae, Tennessee Creek; Aug. 10, larvae, Norton Mill Creek.

Haywood County: June 18, 1939, larva, small branch at Morrison Fish Hatchery.

Swain County (Great Smoky Mountains National Park) 1939—July 11, larva, Indian Creek; July 11, pupa, Deep Creek; Aug. 10, larvae and pupae, Forney Creek; Aug. 10, larvae and pupae, Deep Creek.

Remarks: Not previously reported for Macon, Jackson and Haywood counties.

Family PHILOPOTAMIDAE

Philopotamus distinctus Walker

Macon County: 1939—May 30, larvae, Junction Ball and Shope Creeks; June 22, larvae, Skittles Creek; June 27, larvae, White Oak Creek, Nantahala River; July 26, larvae, Burning Town Creek; July 28, larvae, Big Buck Creek.

Jackson County: June 14, 1939, larvae, Slicken Creek; July 6, 1939, larvae, Tennessee Creek.

Haywood County: June 18, 1939, pupa, branch at Morrison Fish Hatchery.

Transylvania County: June 5, 1939, pupa, Toxaway River.

Swain County (Great Smoky Mountains National Park) July 2, 1939, larvae and pupae, Oconaluftу River; Aug. 13, 1939, larva, Forney Creek.

Remarks: Range extended to Macon, Jackson, Haywood, and Transylvania counties.

Family HYDROPSYCHIDAE

Diplectrona modesta Banks

Macon County: 1938—May 28, larvae, Cullasaja River; June 1, 3, larvae, Big Creek. 1939—May 30, larvae, Junction Ball and Shope Creeks, Dirty John Creek; May 30, larvae and pupae, Jarrett Creek and Nantahala River; June 20, larvae, Big Creek; June 22, pupae, Cullasaja River; June 22, larvae, Skittles Creek; June 23, larvae, Kline's Lake; June 27, larvae, Cold Spring and White Oak Creeks; June 27, pupa, Otter Creek; June 27, nymph and pupa, Choga Creek; July 28, larvae, Big Buck Creek.

Jackson County: 1939—June 14, larva, Slicken Creek; July 12, larvae, tributary of High Hampton Lake; July 20, larvae, Knob Creek.

Swain County (Great Smoky Mountains National Park) July 11, 1939, larva, Indian Creek; Aug. 13, 1939, larvae, Forney Creek.

Remarks: Only previous record for state from Raleigh (Brimley, 1938).

Hydropsyche slossonae Banks

Macon County: 1938—June 3, larvae, Big Creek. 1939—May 30, larvae, Junction Ball and Shope Creeks, Jarrett Creek, Nantahala River; May 30, pupae, Junction Ball and Shope Creeks; June 24, pupae, Lower Cullasaja River; June 27, larvae, White Oak Creek.

Remarks: Previously reported only from Swannanoa (Brimley, 1938).

Hydropsyche sparna Ross

Jackson County: Aug. 28, 1938, pupa, Chattooga River.

Remarks: New record for state. Specimen deposited in Illinois Natural History Survey Collection.

Family ARCTOPSYCHIDAE

Parapsyche cardis Ross

Macon County: 1939—May 30, larvae, Nantahala River.

Jackson County: 1939—June 2, larvae, small waterfall on U. S. Highway #64.

Swain County (Great Smoky Mountains National Park): 1939—July 10, larvae, Bradley Fork; Aug. 13, larva, Forney Creek.

Remarks: Reported only from Smokemont, type locality (Brimley, 1938). Range extended to Macon and Jackson counties.

Family CALAMOCERATIDAE

Heteroplectron gameta Ross

Macon County: 1939—May 26, larva, small stream in Primeval

Forest, Highlands; June 27, larvae, Choga Creek and Nantahala River; July 28, larva, Big Buck Creek.
Jackson County: June 4, 1939, larva, Norton Mill Creek.
Remarks: New record for state.

Family LEPTOCERIDAE

Athripsodes ancylus Vorhies
Swain County (Great Smoky Mountains National Park): Aug. 16, 1939, larva, Deep Creek.
Remarks: New to state.

Family LIMNEPHILIDAE

Stenophylax sonso Milne
Jackson County: 1938—Aug. 17, pupae, Green's Creek; Aug. 19, larvae and pupae, Fowler Creek.
Remarks: New record for state.

Stenophylax lucentulus Betten
Macon County: 1938—June 1, 3, larvae, Big Creek; Aug. 24, pupa, Big Creek.
Remarks: New record for state.

Stenophylax scabripennis Rambur
Jackson County: 1938—Aug. 19, larvae and pupae, Fowler Creek; Aug. 27, adult female, Norton Mill Creek.
Remarks: Reported from Linville as *Pycnopsyche scabripennis* Rambur (Brimley, 1938). Specimens deposited in Illinois Natural History Survey Collection.

Family SERICOSTOMATIDAE

Lepidostoma sp?
Swain County (Great Smoky Mountains National Park): Aug. 13, 1939, pupae, Forney Creek.
Remarks: Genus new to state.

DEPARTMENT OF BIOLOGY,
WESLEYAN COLLEGE,
MACON, GEORGIA.

LITERATURE CITED

BRIMLEY, C. S. 1938. Insects of North Carolina. Publication North Carolina Department of Agriculture, Division of Entomology.

NEEDHAM, J. G., AND P. W. CLAASSEN. 1925. A monograph of the Plecoptera or stoneflies of America North of Mexico. LaFayette, Indiana.

THE CONSORTES OF CERTAIN NORTH CAROLINA BLATTIDS

By Elizabeth Hatcher

INTRODUCTION

Since Hammerschmidt published the first description of an intestinal nematode from *Blatta orientalis* Linn. in 1838, roaches have been found to harbor a number of consortes on which a great deal of work has been done. The nematode inhabitants of the alimentary canal of the blattids have been well summarized and described by Chitwood (1932). Various papers have been published on the protozoa of common roaches, particularly by Kudo (1926, 1926a, 1936), Kudo and Meglitsch (1938), Meglitsch (1938), and Morris (1935). Cleveland and others (1934) have thoroughly investigated the protozoa of the wood-digesting roach, *Cryptocercus punctulatus* Scudder, and shown that these protozoa are symbiotic. Mercier (1908) and Hollande et Favre (1931) investigated the bacteria of the body fat of roaches, but no work has been done, as far as could be discovered, on the bacteria of the fecal matter of common roaches, or on the intestinal bacteria of the wood-digesting roaches.

A study of the animals associated with North Carolina blattids originally began as a comparison of the parasites of two species, the house cockroach, *Periplaneta americana* Linn., and the wood roach, *Parcoblatta uhleriana* (Saussure) with the possibility in mind of working also on bacteria of feces. Later, a number of the wood-feeding roach, *Cryptocercus punctulatus*, were obtained for examination. Inasmuch as the protozoa of the gut of this roach are known to be symbiotic, and the exact status of the inhabitants of the other two species was not determined, it seemed advisable to use in the title the term *consortes* (sing., *consors*), as suggested by Pearse (1939) as a general name for "all types of association, whether injurious, beneficial, or neutral."

Determination of bacteria from fecal matter of *P. americana* seemed to be a problem of practical as well as scientific interest, and investigation of the bacteria of the intestinal contents of *C. punctulatus* also seemed a worthy project, but although bacterial cultures were made and a few species were obtained and identified, lack of time prevented a tabulation of infections of a satisfactory number of specimens.

The cockroaches used were collected from the Biology Building of Duke University, from a junk room in the basement and a cabinet in an office on the first floor. They were kept in a battery jar with a wire cover and fed bits of bread, but for the most part were examined within a few days after capture. The parcoblattids were found under the bark of decaying logs in the Duke Forest, and lived nicely in glass jars with soil and bark. The specimens of *C. punctulatus* were brought from western North Carolina (near Waynesville) where they occur in large numbers under bark of logs. They lived in a battery jar containing soil and bark from the place they were collected.

I wish to express my deep appreciation to Professor A. S. Pearse, who suggested the problem and directed the investigations. I am also indebted to Professor F. A. Wolf for directing the bacteriological work, and to all those members of the Zoology Department staff who offered helpful suggestions and were of aid in many ways.

OBSERVATIONS

Fifteen individuals of each of three species of roaches were examined, with the following numbers infested: *Periplaneta americana*, 11 (73%); *Parcoblatta uhleriana*, 8 (53%); and *Cryptocercus punctulatus* 15 (100%). Four specimens of *P. americana* were examined which for one reason or another could not be included in the foregoing summary, but which contained certain consortes that did not occur in any of the other roaches studied. These were *Leidynema appendiculata* (Leidy), a common nematode in this roach, which occurred at least twice, and *Lophomonas blattarum* (Stein), a flagellate. It is also true that, since these four were all parasitized, the percentage of infested roaches would be raised to about 79% if they were included.

The number of individuals of *Periplaneta americana* infested with various consortes was as follows: *Nyctotherus ovalis*, 6 (40%; number, 10–274; average, 31.3); *Hammerschmidtiella diesingi*, 8 (58%; no., 1–4; ave., 0.93); *Telastoma bulhoesi*, 3 (20%; no., 1–15; ave., 1.2); Telastoma larvae, 1 (6.5%; no., 1; ave. 0.06). The number of roaches which contained Telastoma and Nyctotherus was 2 (13%); Hammerschmidtiella and Nyctotherus, 1 (6.5%); and those with the protozoan and both nematodes, 2 (13%).

In fifteen *Parcoblatta uhleriana* three species of nematodes were found. *Proterellina aurifluus* in 4 individuals (26%; no., 1–3; ave., 0.3); immature nematodes in body cavity in 4 (26%; no., 40–183; ave., 20) and in intestine of 2 (13%; no., 1–2; ave., 0.2); Proterellina larvae in one

roach (6.5%). Hypopi of mites were found in two individuals (13%; no., 1–2; ave., 0.2).

In *Cryptocercus punctulatus* seven genera of flagellates were observed in the number of individuals indicated: Saccinobaculus, 15 (100%); Trichonympha, 14 (93%); Monocercomnoides, 14 (93%); Hexamita, 13 (87%); Barbulonympha, 13 (87%); Eucomonympha, 12 (80%); Prolophomonas, 6 (40%); and Urinympha, 3 (20%).

All the consortes reported in the three species of blattids occurred in the colon, with the exception of external mites and the nematode larvae which were found in the body cavity of Parcoblatta. These larvae were of unusual interest. Specimens sent to Dr. B. G. Chitwood were identified as free-living species, a rhabditid and probably an aphsamidian, and Dr. Chitwood suggested that they may have come, not from the body cavity but from the body surface, gut, or water used in examination. The writer feels sure that they have been reported correctly, however, for when found the minute larvae were in every case hanging tightly on to the body fat and to the *inside* of the plates of exoskeleton, never to the outside. They were always found thus while the gut was still intact. Perhaps they may have entered through the tracheal tubes from their usual habitat in the soil. Those found in the digestive tract must have been ingested with food, or it is possible that they were Proterellina larvae, as they were not identified separately. It is possible also that the mites identified by Dr. A. S. Pearse as hypopi, also entered through the tracheal tubes. These too were found deeply embedded in the body fat.

The protozoa of Cryptocercus were identified from the work of Cleveland (1934). All the genera he reports from roaches from the mountains of the eastern United States were found, with the exception of Idionympha and Leptospironympha. An interesting variation was found in the percentages of each protozoan present in different animals. No attempt was made to estimate percentage of each separate species, but only of genera, although there was a decided variation in species also. In one roach, there were only a few Trichonympha other than *T. parva* Cleveland, which occurred in large numbers. Generally *T. lata* Cleveland was most abundant. There were a number which contained no *Saccinobaculus doroaxostylus* Cleveland at all, while it is doubtful if any lacked *S. minor* Cleveland. This tiny squirming form seemed to dominate nearly every preparation. The total lack, in one roach, of any species that resembled Trichonympha and Barbulonympha is different from the observations of Cleveland, who reported these present in every

specimen. Close examination of this preparation, however, failed to show any but the wormy Saccinobaculus of all assorted sizes and shapes, a few round, twisting *M. globus* Cleveland, and trembling little Hexamita.

All the bacteria found could not be identified in the time available, but a few were made fairly certain: *Micrococcus parvulus* (Veillon and Zuber), *Bacillus albolactis* Migula, and *Acromobacter hyalinum* (Jordan) from the fecal matter of Periplaneta, and *Bacillus subtilis* Cohn from Cryptocercus. The first mentioned occurs in the oral cavity, but it seems reasonable that it may have been picked up in a "junkroom" where men working about the building frequently enter and doubtless spit on the floor. The next two occur in soil and water respectively, and thus might be found almost anywhere. *B. subtilis* is a common inhabitant of soil and would be expected in a wood-feeding animal. Two more species from Cryptocercus could not be identified, and there was not time to make tests for their cellulose-digesting ability.

DISCUSSION

Except for the mites and larval nematodes, all species found have been reported in roaches before (Chitwood 1932, Cleveland 1934, Dobrovolny and Achert 1934, Leidy 1850). It is of interest that not a single consors was found associated with more than one of the three different species of roaches. This may no doubt be correlated with wide differences in habitats and types of food. Periplaneta is essentially a house roach and feeds on almost anything available; probably the chief food of those used in this study was soiled paper and general debris, as well as the bodies of other roaches and feces. Parcoblatta, though living in old logs, feeds not on wood but on larvae of other insects, as well as living and decaying vegetable matter (Blatchly 1920). Cryptocercus finds its nourishment in the wood which it consumes as it burrows through the decaying tree-trunks. If similarity of consortes is indication of close relationship, these three species are surely separated by a wide gap. Cleveland suggests that in view of the protozoa of Cryptocercus and termites, which belong to the same families, either roaches and termites spring from a common ancestor, or else termites are descended from roaches. The present study does not indicate any such relationship. Only one of the roaches shows any similarity of consortes to another, and those of the third are entirely different.

While Cryptocercus appears to be most highly infested of the three species, it will be remembered that its protozoa are entirely symbiotic, while the inhabitants of the other two species are of questionable status.

The roaches seemed without exception to be in good health; they were certainly active, and Dobrovolny and Ackert (1934) report no harm observed in the lining of the gut of roaches heavily infested by oxyurids. It is true that the largest roach examined (40 mm.) contained no consortes, and the average length of the four uninfested was 37 mm., whereas the average length of the 11 which were infested was 34.7. Whether or not this can be significant with so small a number (15) seems doubtful, although the average difference in length is as much as 2.3 mm. The size of Parcoblattas examined was probably not significant, as all examined were immature and wingless.

Perhaps a very useful study might be made on the bacteria of the fecal matter of the common cockroach. Young (1937) has discussed the roach as a carrier of Giardia cysts, and it may as well often deposit bacteria which are harmful. Such an investigation would require a prolonged study. Duncan (1926) demonstrates the presence of a bactericidal principle resembling a weak antiseptic in the alimentary canal of some insects. This if present in roaches would of course limit the number of bacteria.

Though very little seems to have been done on the bacteria of Cryptocercus, Cleveland reports that there is no evidence that they aid the protozoa in the digestion of cellulose, since they are not harmed by methods which remove protozoa, and the roaches do not live after such treatments; he also states that cellulase is formed only in the protozoa. It appears, however, that these bacteria may well bear further investigation.

SUMMARY

1. The consortes of 15 specimens each of three species of blattids (*Periplaneta americana*, *Parcoblatta uhleriana* and *Cryptocercus punctulatus*) from North Carolina were studied. A few bacterial cultures were made from fecal matter of Periplaneta and of intestinal contents of Cryptocercus.

2. No consors was found associated with more than one of the three species of roaches studied. Periplaneta harbors a protozoan, *Nyctotherus ovalis* Leidy, in large numbers, and two oxyurids, *Hammerschmidtiella diesingi* (Hammerschmidt) and *Telastoma bulhoesi* (Magalhoes). *Leidynema appendiculata*, another oxyurid, and *Lophomonas blattarum*, a flagellate, were also observed. Parcoblatta harbors *Protrellina aurifluus* in the colon, and nematode larvae and mites occur in the body fat, perhaps having gained entrance through the tracheal tubes. Eight of the ten genera of symbiotic protozoa which have been reported as

existing in Cryptocercus were found. Of these Saccinobaculus was by far the most abundant.

3. *Micrococcus parvulus*, *Bacillus albolactis*, and *Achromobacter hyalinum* were found in the fecal matter of Periplaneta. *B. subtilis* and at least two species of bacteria which were not identified occur in the colon of Cryptocercus.

ZOOLOGY DEPARTMENT,
DUKE UNIVERSITY,
DURHAM, N. C.

LITERATURE CITED

BLATCHLEY, W. S. 1920. Orthoptera of Northeastern America. Indianapolis, 1–784.

CHITWOOD, B. G. 1932. A synopsis of the nematodes parasitic in insects of the family Blattidae. Zeitschr. für Parasitenkunde **5**: 14–50.

CLEVELAND, L. R., AND OTHERS. 1934. The wood-feeding roach Cryptocercus, its protozoa, and the symbiosis between the protozoa and the roach. Mem. Amer. Acad. of Arts and Sci. **17**: 185–342.

DOBROVOLNY, C. G., AND J. E. ACKERT. 1934. The life history of *Leidynema appendiculata* (Leidy), a nematode of cockroaches. Parasitology **26**: 468–480.

DUNCAN, J. T. 1926. On a bactericidal principle present in the alimentary canal of insects and arachnids. Parasitology **18**: 238–252.

HOLLANDE, A-CH., ET R. FAVRE. 1931. La structure cytologique de *Blattabacterium cuenoti* (Mercier) n. g. symbiote der tissu adipeux des Blattides. Compt. Rend. Soc. Biol. **107**: 784–786.

KUDO, R. R. 1926. Observations of *Endamoeba blattae*. Amer. J. Hygiene **6**: 139–152.

1926a. Observations of *Lophomonas blattarum*, a flagellate inhabiting the colon of the cockroach, *Blatta orientalis*. Arch. Protistenkunde **53**: 191–214.

1936. Studies on *Nyctotherus ovalis* Leidy, with special reference to its nuclear structure. *Ibid.* **87**: 10–42.

KUDO, R. R., AND P. A. MEGLITSCH. 1938. On *Balantidium praenucleatum* n. sp. inhabiting the colon of *Blatta orientalis*. *Ibid.* **91**: 111–124.

LEIDY, J. 1850. Two new species of infusorial entozoa. Proc. Phila. Acad. Nat. Sci. **5**: 100.

MEGLITSCH. P. A. 1938. Cytological observations on *Endamoeba blattae* from the cockroach, *Blatta orientalis*. Abstr. of thesis. Univ. Illinois, Urbana, 1–10.

MERCIER, L. 1908. Recherches sur les bacteroides des Blattides. Arch. Protistenkunde **9**: 346–358.

MORRIS, S. 1935. Studies of *Endamoeba blattae*. Jour. Morphol. **59**: 225–256.

PEARSE, A. S. 1939. Animal ecology. New York. xii + 642.

YOUNG, M. D. 1937. Cockroaches as carriers of Giardia cysts. Jour. Parasitol. **23**: 102–103.

NEW CRUSTACEAN RECORDS FOR THE CAROLINAS AND FLORIDA

By G. Robert Lunz, Jr.

In the past few years four species of macruran, two anomuran, and four brachyuran crustacea have been added to the collection of The Charleston Museum from localities outside the range commonly reported for these species, or from localities within the range of distribution but not definitely known to be frequented by the species.

MACRURAN CRUSTACEA

Leptochela serratorbita Bate

Type locality: St. Thomas, Virgin Islands.
Distribution: Extreme southern Florida to the Virgin Islands, with a single specimen being recorded for Beaufort, N. C., by the U. S. National Museum.
Additional record: A single specimen (Chas. Mus. No. 35.246) was picked up by the author on a sand bar in Charleston Harbor in a small pool left by the receding tide on July 19, 1935.

Thor paschalis (Heller)

Type locality: The Red Sea.
Distribution: Several localities in the Indian Ocean; Bermuda; West Indies; extreme southern Florida; Yucatan; and a single specimen from Beaufort, N. C., recorded by the U. S. National Museum.
Additional record: While dredging in Beaufort River between Beaufort, S. C., and Port Royal, the author collected a single specimen (Chas. Mus. No. 35.129.2) on May 3, 1935.

Brachycarpus biunguiculatus (Lucas)

Type locality: Coast of Algeria.
Distribution: The Mediterranean. The West Indies, Bahamas, and Bermuda. In the Pacific from Clipperton Island off west coast of Mexico.
Additional records: Thanks to the activities of Mr. C. J. D. Behrens of

the Lighthouse Tender "Cypress" (Sixth Lighthouse District), we have several specimens of *Brachycarpus* in the collection:

Three specimens (Chas. Mus. No. 38.209.3). 47 miles south southeast of Cape Canaveral, Florida, from Bethel Shoal sea buoy on May 12, 1938.

One specimen (Chas. Mus. No. 38.209.1). 15 miles northeast of Cape Canaveral, Florida, from Hetzel Shoal sea buoy on May 16, 1938.

One specimen (Chas. Mus. No. 37.223). From sea buoy 1 mile outside Ft. Pierce Inlet, Florida, on January 13, 1937.

One specimen (Chas. Mus. No. 37.224) from sea buoy 2AFP on the outer end of Frying Pan Shoal off Cape Fear, North Carolina, on May 4, 1937.

Macrobrachium ohionis (Smith)

Type locality: Ohio River at Cannelton, Indiana.
Distribution: Middle and lower Mississippi River; North Carolina and Georgia.
Additional records: There are several specimens and records in The Charleston Museum of this species from the Cooper River above Charleston where it has been taken regularly by T. K. Ellis.

I have also examined a number of specimens taken in the Edisto River in South Carolina from Pine Landing to Hart's Bluff. The specimens were taken by John C. Pearson and were retained by him.

Mr. Pearson also has a specimen taken at Avoca, North Carolina, in April 1938. He reports having examined two more specimens from the same locality taken by commercial fishermen in a seine.

ANOMURAN CRUSTACEA

Emerita benedicti Schmitt

Type locality: Tampa, Florida.
Distribution: Type locality only.
Additional records: Two specimens (Chas. Mus. No. 35.103.19) taken in a dredge in Folly River, S. C., by the author on April 24, 1935.

Three specimens—including one ovigerous female—(Chas. Mus. No. 37.135.2) taken on Edisto Island, S. C., by H. M. Rutledge on June 17, 1937.

Several specimens (Chas. Mus. No. 38.190.1) taken by the same collector at the same locality on July 25, 1938.

One specimen (retained by collector) dredged in 2 fathoms of water

on shell bottom about 1 mile off Edisto Island, S. C., by T. K. Ellis on January 7, 1939.

Euceramus praelongus Stimpson

Type locality: Beaufort, North Carolina.
Distribution: Chesapeake Bay, North Carolina, and Florida.
Additional records: Twenty-eight specimens (Chas. Mus. No. 36.5.2) taken by the author on Isle of Palms beach near Charleston, S. C., on January 7, 1936.

One specimen dredged in three fathoms of water in the mouth of the South Edisto River, S. C., by H. M. Rutledge on July 12, 1937. Specimen in the collection of Edisto State Park.

Two specimens (retained by collector) taken in dredge in two fathoms of water about 1 mile off Edisto Island, S. C., by T. K. Ellis on January 7, 1939.

BRACHYURAN CRUSTACEA

Dromidia antillensis Stimpson

Type locality: St. Thomas, V.I., Key Biscayne, and Tortugas, Florida.
Distribution: North Carolina at Cape Hatteras, Beaufort, and Cape Fear. Also Bermuda, southern Florida, Gulf of Mexico, and Brazil.
Additional records: One specimen (Chas. Mus. No. 39.145) from sea buoy No. C-2 off Charleston Harbor, S. C., taken by C. J. D. Behrens (U.S.L.H.T. "Cypress") on June 27, 1939.

One mangled specimen (Chas. Mus. No. 35. 18.2) from sea buoy at St. Augustine, Florida, by T. B. Christiansen (U.S.L.H.T. "Cypress") on January 25, 1935.

Hypoconcha sabulosa (Herbst)

Type locality: "Africa (probably error)"*
Distribution: North Carolina, Florida Keys, and West Indies.
Additional records: One specimen (Chas. Mus. No. 36. 107.1) from Magnolia Beach (near Myrtle Beach), South Carolina. Taken on June 1, 1936, by S. W. Norris.

One specimen (Chas. Mus. No. 38. 285) from Pawley's Island (near Myrtle Beach), South Carolina. Taken by J. Kaminski during the summer of 1938.

* Rathbun, M. J. 1937. Bull. 166 U. S. Nat. Mus., p. 45.

Ebalia cariosa (Stimpson)

Type locality: Beaufort, North Carolina.
Distribution: Beaufort, N. C.; Florida; Jamaica; Brazil.
Additional records: One specimen (Chas. Mus. No. 35. 130.9) dredged in 3 fathoms of water in Beaufort River between Beaufort, South Carolina, and Port Royal. Taken by the author on May 3, 1935.

Pilumnus marshi Rathbun

Type locality: St. Thomas, Virgin Islands.
Distribution: Tortugas, Florida, and St. Thomas, V.I.
Additional records: One specimen (now in U. S. National Museum) taken off sea buoy at Lake Worth, Florida, by T. B. Christiansen (U.S.L.H.T. "Cypress") in May 1936.

One specimen (Chas. Mus. No. 38. 228) taken from sea buoy 2 FP on Frying Pan Shoal off Cape Fear, N. C., on May 4, 1938, by C. J. D. Behrens (U.S.L.H.T. "Cypress").

CHARLESTON MUSEUM,
CHARLESTON, S. C.

A TAXONOMIC REVISION OF THE GENUS SIPHONYCHIA*

By Earl L. Core

One Text Figure

While engaged in the examination of a few sheets of certain Corrigiolaceae of the eastern United States, I became impressed by the apparent lack of organization in most herbaria of material belonging in the genus *Siphonychia* and related groups. The present paper arose through an effort to correct this situation with the least possible reshuffling of nomenclature and may, therefore, be regarded as a most conservative treatment.

The name *Siphonychia*, derived from the Greek σίφων, a tube, that is, an *Anychia* with a tubular calyx, was first used for these plants by Torrey and Gray in 1838.[1] But one species, *S. americana*, was included in this first treatment.

Three additional species were characterized by Chapman in 1860, viz., *S. diffusa*, *S. erecta*, and *S. Rugelii*.[2] In 1897 Small published the description of *S. corymbosa*[3] and in 1903 the same author characterized *S. pauciflora*.[4]

The genus retained its integrity until 1898, when Small separated *S. Rugelii*, a species with the "flowers subtended by indurated bracts at maturity," under the genus *Gibbesia*.[5] Five years later the same author separated *S. erecta* and *S. corymbosa*, species without a mucro on the sepals, under the genus *Odontonychia*.[6] A third species was added to this genus in 1933, when Small published, although without Latin diagnosis, his characterization of *O. interior*.[7]

The relatively minor characteristics on the basis of which Small separated *Gibbesia* and *Odontonychia* seem to the present author to be

* Contribution No. 10 from the Herbarium of West Virginia University.

[1] Fl. N. Am. 1: 173.

[2] Fl. S. U. S., p. 47.

[3] Bull. Torr. Bot. Club 24: 337.

[4] Fl. S. E. U. S., p. 402.

[5] Bull. Torr. Bot. Club 25: 621.

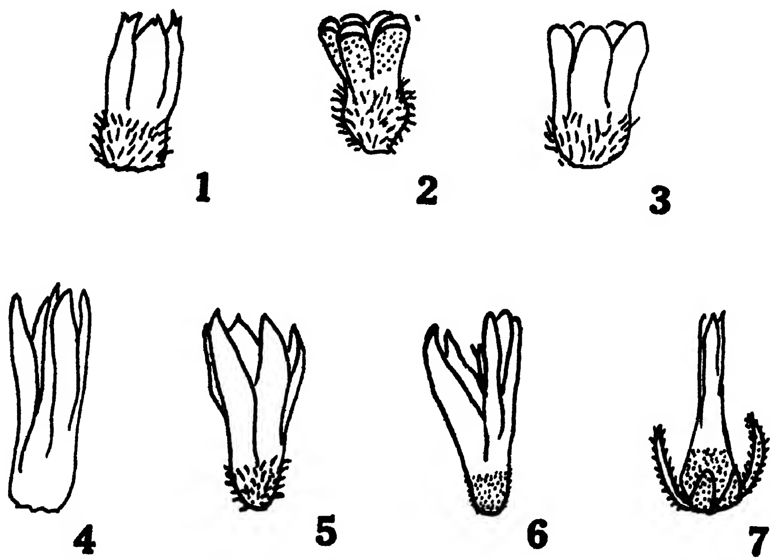
[6] Fl. S. E. U. S., p. 401.

[7] Man. S. E. Fl., p. 483.

scarcely of generic value, and in the present treatment the original genus is retained intact.

The group, finally including seven species in all, has a rather restricted geographical distribution, being found only on the coastal plain from South Carolina to Florida and Louisiana, occurring principally in dry pinelands, oak woods, scrub, or on sand dunes.

The species are annual, biennial, or perennial herbs with radially prostrate, diffuse, or erect stems and branches. The leaves are small,



Flowers of Siphonychia

1. *S. diffusa;* 2. *S. americana;* 3. *S. pauciflora;* 4. *S. erecta;* 5. *S. interior;* 6. *S. corymbosa;* 7. *S. Rugelii.*

stipulate, narrow, entire, sometimes somewhat fleshy. In some species the blades are more or less curved.

The perfect flowers are in cymes which are several- to many-flowered, corymbose or glomerulate, compact or open. The calyx is composed of 4 or 5 sepals which are often cuspidate or mucronate at the apex, petaloid above, coherent into a tube below. The corolla is wanting. The androecium consists of 4 or 5 stamens inserted on the throat of the calyx. The style is long and filiform, minutely bifid at the apex. The utricle is included in the calyx-tube.

The genus *Siphonychia*, in the arrangement of Engler and Prantl,[8] was included in the Caryophyllaceae, in the tribe Alsinoideae-Paronychieae. In the Bentham and Hooker sequence it is separated into a separate family, the Illecebraceae,[9] although Reichenbach much earlier had set up the family Corrigiolaceae.[10] This family is separated from the Caryophyllaceae through its fruit, a 1-seeded utricle, contrasting with the several- or many-seeded pod of the latter family. *Paronychia*, the genus most closely related to *Siphonychia*, has its sepals distinct or only slightly united at the base, and stamens that are borne on the base of the sepals.

MATERIAL STUDIED

Material in *Siphonychia* from several of the larger herbaria in the United States has been studied in the preparation of this paper. The following abbreviations have been used to designate the various institutions: G, Gray Herbarium; NY, New York Botanical Garden; US, United States National Herbarium; P, Philadelphia Academy of Natural Sciences; F, Field Museum of Natural History; B, Brooklyn Botanic Garden; Mich, University of Michigan; M, Missouri Botanical Garden; W. Va., West Virginia University; C, Carnegie Museum; D, Duke University.

The assistance rendered by my friend Dr. Harold N. Moldenke, of the New York Botanical Garden, in copying out literature not available to me at Morgantown, is hereby acknowledged with deep gratitude. I also wish to express my appreciation to the heads of the various institutions mentioned above, for their kindness in making available the material for this study.

SIPHONYCHIA Torrey & Gray, Fl. N. Am. **1**: 173. 1838.

Buinalis O. Ktze., Rev. Gen. **2**: 534. 1891. *Forcipella* Small, Bull. Torr. Bot. Club **25**: 150. 1898, not Baillon ex Engler & Prantl, Nat. Pflanzenfam. IV, Abt. 3b: 343. 1895. *Gibbesia* Bull. Torr. Bot. Club **25**: 621. 1898. *Odontonychia* Small, Fl. S. E. U. S., p. 401. 1903.

[8] Nat. Pflanzenfam. 3, 1b: 91. 1889.
[9] Gen. Plant. f. 3. 16.
[10] Moessl. Handb. 1:[1] 51. 1827.

KEY TO THE SPECIES

1. Flowers subtended by herbaceous bracts.
 2. Sepals short and broad, mucronate.
  3. Sepals narrowed at the tip............................1. *S. diffusa.*
  3. Sepals broadened at the tip.
   4. Cymes many-flowered; bracts minute....................2. *S. americana.*
   4. Cymes few-flowered; bracts foliaceous..................3. *S. pauciflora.*
 2. Sepals long and narrow, not mucronate.
  3. Stems glabrous..4. *S. erecta.*
  3. Stems pubescent.
   4. Calyx tube bristly-pubescent..............................5. *S. interior.*
   4. Calyx tube minutely pubescent.......................6. *S. corymbosa.*
1. Flowers subtended by indurated bracts......................7. *S. Rugelii.*

1. **S. diffusa** Chapm., Fl. S. U. S., p. 47. 1860.
Buinalis diffusa O. Ktze., Rev. Gen. **2**: 534. 1891.

Minutely pale-pubescent; stems prostrate, 1–6 dm. long, diffusely branched; leaves oblanceolate, linear-oblanceolate or linear-spatulate to almost linear, 0.5–2.5 cm. long, obtuse; stipules conspicuous, on young plants half as long as the leaves, at length two-parted; flowers over 1.5 mm. long, in compact, rectangular terminal cymes; sepals ovate to linear, slightly concave and mucronate at the apex, the tube bristly with hooked hairs.

Type Locality—Florida.

Distribution—FLORIDA: Apalachicola, Franklin Co., *Chapman* 4598 (NY, G); Cedar Keys, Levy Co., *Palmer* (NY, G); Eustis, Lake Co., *Nash* 1167 (NY, G, P, F); between Avon Park and Sebring, De Soto Co., *Small, Small, & DeWinkeler* 11491 (NY); between Quincy and Wetumka, Gadsden Co., *Small, Small, & DeWinkeler* 11395 (NY); Oakland, Orange Co., *Lewton* (NY, F); Dunellon, Marion Co., *Curtiss* 6659 (NY, G, B, P); Tallahassee, Leon Co., *Berg* (NY); Walton Co., *Curtiss* (NY); Pensacola, Escambia Co., *Curtiss* 6921 (NY, G, P); Lynn Haven, Bay Co., *Billington* (Mich); Madison Co., *Hitchcock* 1558 (F); Columbia Co., *Hitchcock* 1559 (F). ALABAMA: "Alabama," *Buckley* (G). LOUISIANA: "Sandy shores of Bayou," *Mohr* (NY).

2. **S. americana** T. & G., Fl. N. Am. **1**: 173. 1838.
Paronychia americana Fenzl ex Walp., Rep. **1**: 262. 1842.
Paronychia urceolata Shuttlw. ex Chapm., Fl. S. U. S., p. 47. 1860.

Stems 2–8 dm. long, prostrate, diffuse, minutely retrorse-pubescent when young, obscurely pubescent or glabrous in age; leaves linear-oblanceolate to spatulate, 0.5–2 cm. long, minutely pubescent and ciliate, narrowed at the base, the radical ones larger and crowded; stip-

ules small; flowers obovate, scarcely 1.5 mm. long, solitary in the forks of the stem and clustered in cymes at the end of the branches; sepals white above, obovate, rounded and incurved at the apex, the tube bristly with hooked hairs.

Type Locality—"Florida to South Carolina and westward."

Distribution—SOUTH CAROLINA: "South Carolina," *Nuttall* (NY). GEORGIA: Augusta, Richmond Co., *Cuthbert* 772 (NY); Little River, s.w. of Tifton, Berrien Co., *Harper* 1696 (NY, F, G, B); Seventeen Mile Creek, Coffee Co., *Harper* 700 (NY). FLORIDA: Alva, Lee Co., *Hitchcock* 285 (NY, G, F); near Snapper Creek, Dade Co., *Small* 11589 (NY); Leon Co., *Chapman* (NY); St. Augustine, St. Johns Co., *Reynolds* (NY, B, Mich); Jupiter, Palm Beach Co., *Curtiss* 5546 (NY, G, B, F); Merritt's Island, Indian River, Brevard Co., *Curtiss* 342 (NY, G, B, F); 5 mi. s. of Daytona Beach, Volusia Co., *Small, Small, & DeWinkeler* 10570 (G); Jessamine, Pasco Co., *Barnhart* 2605 (F); Rosewood, Levy Co., *Garber* (F).

3. **S. pauciflora** Small, Fl. S. E. U. S., p. 402. 1903.

Stems and branches 1–6 dm. long, obscurely pubescent or glabrous in age; leaves linear-oblanceolate to spatulate, 0.5–2.5 cm. long; cymes with foliaceous bracts, few-flowered; flowers about 1.5 mm. long; sepals obovate-cuneate, with a broad dilated hood at the inflexed apex and an obscure cusp.

Type Locality—"In sand or sandy soil, Georgia and Florida."

Distribution—GEORGIA: Oconee River, opposite Dublin, Laurens Co., *Harper* 1353 (NY, G); Augusta, Richmond Co., *Cuthbert* 166 (NY); Big Lott's Creek, Bulloch Co., *Harper* 967 (NY, G). FLORIDA: Snapper Creek, Dade Co., *Small* 11591 (NY); "ad fluv. Ocklockonne prope Tallahassee," Leon Co., *Rugel* in 1843 (NY); New Smyrna, Volusia Co., *Small* 11566 (NY); along Suwanee River, near Old Town, Lafayette Co., *Small, DeWinkeler, & Mosier* 11322 (NY); West Palm Beach, Palm Beach Co., *Mrs. Deam* 1711 (G); "prope St. Augustine," St. Johns Co., *Rugel* in 1848 (G); Georgiana, Brevard Co., *Smith* (P); St. Petersburg, Pinellas Co., *Williamson* (P); Rosewood, *Garber* (F); Citrus Co., *Hitchcock* 1560 (F).

4. **S. erecta** Chapm., Fl. S. U. S., p. 47. 1860.

Buinalis erecta O. Ktze., Rev. Gen. **2**: 534. 1891.

Odontonychia erecta Small, Fl. S. E. U. S., p. 401. 1903.

Stems smooth, glaucous, clustered, erect, 1–4 dm. tall; leaves erect, spatulate, linear-oblanceolate or linear, 0.5–3 cm. long, those of the

barren stems imbricated; stipules silvery, very conspicuous against the stem and leaves; cymes compound, rectangular, compact; flowers nearly or quite 3 mm. long; sepals lanceolate, smooth, acutish, sometimes obscurely mucronate at the apex; calyx tube smooth and furrowed; utricle about 1 mm. long.

Type Locality—Sands along the west coast of Florida.

Distribution—FLORIDA: Hurricane Island, St. Andrews Bay, Bay Co., *Banker* 3669 (B); Santa Rosa Island, *Tracy* 6409 (NY); "East Florida," *Buckley* (NY). ALABAMA: Perdido, Baldwin Co., *Tracy* 8689 (NY, G, F); Mobile, Mobile Co., *Mohr* (NY, B, F).

5. **S. interior** Core n. comb.

Odontonychia interior Small, Man. S. E. Fl., p. 483. 1933.

Plant closely brown-pubescent and often somewhat viscid, the branches spreading, 1–4 dm. long; leaves spatulate to linear, 0.5–2.5 cm. long, relatively coarse-pubescent; cymes open, diffuse; flowers becoming 2.5 mm. long; sepals lanceolate, acute, much longer than the calyx tube; utricle less than 1 mm. long.

Type Locality—"Along the Suwanee River, e. of Old Town, Fla., *Small, Small, & DeWinkeler*, No. 11465, in herb. N. Y. B. G."

Distribution—FLORIDA: along Suwanee River east of Old Town, Lafayette Co., *Small, Small, & DeWinkeler* 11465 (NY); pinelands west of Lake City, Columbia Co., *Small, Small, & DeWinkeler* 11372 (NY); Citrus Co., *Hitchcock* 1553 (F); Suwanee Co., *Hitchcock* 1552 (F); near mouth of Spring Warrior River, *Small, Small, & DeWinkeler* 11451 (NY, G). GEORGIA: Flint River at West Bainbridge, Decatur Co., *Harper* 1234 (NY, G).

6. **S. corymbosa** Small, Bull. Torr. Bot. Club **24:** 337. 1897.

Odontonychia corymbosa Small, Fl. S. E. U. S., p. 402. 1903.

Plant minutely grayish-pubescent, the branches erect, 1–3 dm. tall; leaves oblanceolate to elliptic or linear, 0.5–1.5 cm. long, minutely pubescent; cymes aggregated in a compact terminal corymb; flowers scarcely 2.5 mm. long; sepals ovate, obtuse, slightly longer than the hypanthium; utricle about 1 mm. in diameter.

Type Locality—"The original specimens were collected by Professor L. M. Underwood on Ship Island, on the coast of Mississippi, in June, 1896."

Distribution—FLORIDA: Port St. Joe, Calhoun Co., *Small, Small, & DeWinkeler* 11422 (NY); East Pass, Franklin Co., *Tracy* 6408 (NY);

High Springs, Alachua Co., *Wiegand & Manning* 1164 (G). ALABAMA: Mobile, Mobile Co., *Mohr* (P, F). MISSISSIPPI: Ship Island, Biloxi, Harrison Co., *Earle & Underwood* (NY, F); Cat Island, Harrison Co., *Lloyd & Tracy* 76 (F, NY); Horn Island, Jackson Co., *Tracy* 7652 (NY, G, F); Ocean Springs, Jackson Co., *Tracy* (NY).

7. **S. Rugelii** Chapm., Fl. S. U. S., p. 47. 1860.

Paronychia Rugelii Shuttlw. ex Chapm., Fl. S. U. S., p. 47. 1860, in synon.

Buinalis Rugelii O. Ktze., Rev. Gen. **2**: 534. 1891.

Forcipella Rugelii Small, Bull. Torr. Bot. **25**: 150. 1898.

Gibbesia Rugelii Small, Bull. Torr. Bot. Club **25**: 621. 1898.

Annual; stem erect, 1–5 dm. tall, rather closely pale pubescent, at length effusely branched; leaves narrowly oblanceolate, linear-oblanceolate, or nearly linear, 1–3 cm. long, pubescent; stipules one-fourth to one-third as long as the leaves, at length two-parted; cymes numerous, terminal, rather loosely several- to many-flowered; calyx tube short, pubescent, the sepals linear-lanceolate, about 2 mm. long, conspicuously mucronate, white; utricle included.

Type Locality—Florida.

Dr. Chapman was dissatisfied with his original disposition of this species and in the third edition of his Southern Flora he transferred it to the genus *Paronychia*. Small, in 1898, erected for this species the new genus *Forcipella*, a name which was a diminutive of *forceps*, referring to the clamp-like involucels. This name, however, had been employed earlier by Baillon for a genus in the Acanthaceae, and later in the same year (1898), Dr. Small changed the name of the genus to *Gibbesia*, in honor of Prof. Lewis R. Gibbes, of Charleston, S. C.

Distribution—GEORGIA: Flint River at West Bainbridge, Decatur Co., *Harper* 1234 (F); Little Ocmulgee River, Montgomery Co., *Harper* 1990 (NY, G, F); Olympia, Lowndes Co., *Harper* 1596 (NY); Ohoopee River, Shepherd's Bridge, Tatnall Co., *Leeds* 1722 (P, D). FLORIDA: "ad fluv. Whittlecouchy prope Camp Island," *Rugel* in 1848 (NY, G); Suwanee, Suwanee Co., *Williamson* (P).

DEPARTMENT OF BOTANY,
WEST VIRGINIA UNIVERSITY,
MORGANTOWN, W. VA.

OBSERVATIONS ON A NEW SPECIES OF THRAUSTOTHECA*

By Mary Williams Ward

Plate 31

During the winter of 1939 the writer became interested in a study of aquatic Phycomycetes obtained from soil and water samples in the vicinity of Burgaw, North Carolina (alt. 50–60 ft.; soil soft sandy loam). These samples were brought into the laboratory where a number of species of the Saprolegniaceae and a few Chytrids were isolated.

The first systematic study of water molds occurring in the soil was made in this laboratory by Harvey (1925), and since then numerous other investigators have found water molds in soil collections.

PROCEDURE

The author collected numerous samples of soil from near the surface of the ground in the vicinity of Burgaw, North Carolina. Also samples of water containing sediment were taken from various pools, streams, ponds, and springs in the same general collecting region. These collections were brought to the laboratory at Chapel Hill and placed in Petri dishes. To these were added a small amount of tap water, three or four halves of boiled hemp seed, and several pieces of cooked grass. The dishes were covered and allowed to stand for two or three days without being examined. After two days a piece of grass from each culture was transferred to a sterile Petri dish, washed, and examined for Chytrids. The other grass and the hemp seed in these original cultures were allowed to remain for four or five days to await the growth of any fungus which might appear later. At the end of this period, the remaining pieces of grass and the hemp seed with their fungal growth were transferred to sterile Petri dishes, washed, and the fungus species identified whenever possible. A single hyphal tip of any water mold which appeared to be interesting was transferred to a plate of cornmeal agar or to a plate of maltose-peptone #5 (Couch 1932) to

* Material in this paper taken from a thesis presented in partial fulfillment of the requirements for the master's degree in the Department of Botany, University of North Carolina.

obtain pure cultures. After 2 or 3 days' growth, small squares of agar containing tips of hyphae which were free from bacteria were cut out and transferred to sterile Petri dishes. A small piece of hemp seed was placed on each of these blocks of agar and a little sterile water added to each dish. Usually, after one day the hemp seed was completely surrounded by a vigorous young growth of the plant mycelium.

RESULTS

A record of all identifications and the number of times collected from both soil and water is shown in the tables given below.

Isolations from Soil Collections

Species	*No. of times collected*
Aplanes Treleaseanus	4
Achlya racemosa	1
Achlya colorata	2
Achlya americana	1
Achlya proliferoides	3
Achlya flagellata	3
Achlya imperfecta	1
Achlya apiculata	6
Achlya megasperma	2
Achlya prolifera	1
Achlya recurva	1
Achlya (sterile)	2
Thraustotheca n. sp.	2
Dictyuchus sp.	1
Leptolegnia sp.	1
Allomyces arbuscula	4
Olpidiopsis fusiformis	1
Olpidiopsis minor	1
Olpidiopsis varians	6
Rhizophidium carpophilum	1
Rhizophidium multiporum on *Achlya flagellata*	1
Rhizidiomyces apophysatus	1

Isolations from Water Collections

Species	*No. of times collected*
Pythiopsis sp.	3
Saprolegnia delica	1
Saprolegnia litoralis	2
Aplanes Treleaseanus	5
Achlya colorata	2
Achlya proliferoides	12
Achlya flagellata	5
Achlya imperfecta	6

Species	*No. of times collected*
Achlya apiculata	2
Achlya (sterile)	2
Thraustotheca n. sp.	7
Dictyuchus sp.	20
Aphanomyces stellatus	1
Aphanomyces sp.	1
Allomyces arbuscula	1
Pythium sp.	3
Olpidiopsis varians	1
Rhizophidium carpophilum on threads of *Achlya* sp.	2
Rhizidiomyces apophysatus on *Achlya flagellata*	5
Rhizopidium multiporum	2
Endochytrium sp.	1
Rhizophlyctis rosea	1

In the table given above, as is indicated, a new species of *Thraustotheca* and a Chytrid, *Rhizophlyctis rosea*, previously incompletely described, have been obtained. A complete description of this new *Thraustotheca* is given below; the development of *Rhizophlyctis rosea* will be discussed in a separate paper.

The following description was drawn from single spore cultures on hemp seed in distilled water which had been filtered through animal charcoal and sterilized.

Thraustotheca irregularis Coker and Ward n. sp.

Homothallic; growth dense and rapid on boiled hemp seed; hyphae often over 2 cm. long, 12–71 μ thick near the base, rarely 128.5 μ; mostly branching, seldom unbranched; tips mostly somewhat pointed and less granular than the remainder of the hyphae. Sporangia abundant; the first ones borne on the ends of the main hyphae, with few exceptions of the modified *Achlya* type, rather stout and regular to slender and irregular, subcylindrical or usually thicker near tip or center. Wall thin and delicate and eventually disappearing in part, more often entirely disappearing some time after emptying; the spores escaping through an inconspicuous apical papilla and encysting at the tip in the form of a hollow sphere, soon emerging and swimming away in the *Achlya* form. Encystment of most of the spores sometimes taking place within the sporangium, but a true dictyosporangium is never formed. Later sporangia of the *Thraustotheca* type borne singly or in large clusters up to 4 or 5 on the ends of hyphae, irregular in form, often curved or very crooked and very often forked; spore mass usually breaking away as a whole or in part; spores encysting within, spherical or subangular, the sporangial wall soon disappearing; spores leaving a false net after emerging. Gemmae usually abundant, spherical, pyriform, or flask-shaped, oval, clavate, or somewhat fusiform, intercalary or borne on the ends of hyphae, often in chains of two or three, some-

times as many as five; cylindrical gemmae also formed by the segmentation of the hyphae after sporangia have emptied, breaking off in some cases just as do the sporangia. Oogonia borne on short lateral stalks, the length of which may not equal the diameter of the oogonia, rarely on the ends of main hyphae or intercalary, smooth-walled, spherical to oval, very regular in size, 21.4–71.5 μ, mostly about 50 μ, wall rather thin, pitted or occasionally unpitted. Eggs 16.6–38.3 μ thick, 1–9 in an oogonium, usually 3–6, eccentric with a single, large lateral oil drop when mature. Antheridia on all oogonia that reach maturity, diclinous, a large number of antheridial branches commonly found on each oogonium, twining about it; antheridia simple or branched and usually finger-like in shape, being applied to the oogonia by their sides or ends.

Collected 9 times from soil and water samples taken in the vicinity of Burgaw, North Carolina, January 13–14, 1939.

From the other three species of water molds that produce sporangia of both the *Achlya* and *Thraustotheca* types, this species is distinguished as follows:

From *T. primoachlya* by the smooth oogonia, abundant gemmae, and diclinous antheridia; from *A. dubia* by the hyaline oogonial wall, the entire contents of the sporangium forming spores, and the absence of true dictyosporangia; from *Brevilegnia bispora* by the much larger oogonia with several eggs and the diclinous antheridia. One of its most peculiar characteristics is the formation of sexual organs only in poorly nourished cultures.

In the first isolations of this fungus no sexual organs appeared, thus suggesting that it might be heterothallic. Since this species had been isolated from several collections, crosses were made according to the method described by Raper (1936) to determine whether or not compatible strains had been found. Isolations of this species were also crossed with sterile Achlyas. These crosses all failed to produce oogonia and antheridia. However, in several collections, miniature cultures growing on small particles of hemp seed were found producing oogonia and antheridia in abundance. Because of the fact that the antheridia were diclinous further experiments were necessary to prove that this species was not heterothallic. Consequently, numerous single spore and single hyphal cultures were made. In addition, other cultures were made by cutting off oogonia to which no antheridia were attached, and by cutting off antheridial branches which had not attached themselves to oogonia. All of these were planted separately on maltose-peptone #5 agar. Transfer cultures from these isolations were placed on halves of hemp seed in Petri dishes and to each a small amount of

distilled water was added. On these cultures no oogonia or antheridia were produced. When small bits of hemp seed or when termites which had been preserved in alcohol and washed in distilled water were added to these cultures, the production of antheridia and oogonia occurred in almost every instance. Therefore, it was determined that this species is homothallic, but that it must be starved before oogonia and antheridia are produced.

To show further that a shortage of food material initiates sexual reproduction, the following cultures were made: in one Petri dish with part of an old culture that had produced oogonia, were placed four radicles of hemp seed, one of which was very small. The length of the hyphae on the three large radicles after two days extended 8–10 mm. from the radicle. None of these three cultures gave any evidence of sexual reproduction. The growth on the smaller piece was much less extensive, measuring only about 2 mm. from the radicle. On this culture, oogonia and antheridia were abundant. In another Petri dish with part of a culture that was producing oogonia, four cotyledons of hemp seed were placed. The length of the hyphae on these four cotyledons after two days was from 6–10 mm. from the cotyledon and no sex organs were produced. However, a small part of one cotyledon which had broken off produced hyphae which measured only 5 mm. and sex organs were produced in abundance. Four pieces of corn embryo were placed in a third Petri dish containing an old oogonial culture. After two days' growth, the hyphae of all these cultures measured approximately 10 mm. Three cultures were very vigorous and produced no sexual organs, while the remaining culture was rather delicate and produced few sex organs. Particles of corn endosperm were also placed in a Petri dish with an old culture which had produced oogonia. After two days' growth the hyphae on the three large particles of endosperm measured from 5–6 mm. in length. None of these three cultures gave any evidence of sexual reproduction. The hyphae of the smaller culture, however, measured only three mm. in length; on this culture oogonia and antheridia were abundant.

When transfer cultures were placed on small bits of hemp seed, on termites, on house flies, or on wasps, without first obtaining a vigorous growth on larger pieces of hemp seed, no oogonia or antheridia were produced.

The remarkable regularity in the appearance and succession of two kinds of sporangia (fig. 1) and the fact that the later sporangia are frequently curved and often more or less in clusters at the ends of

hyphae (fig. 10), indicated a resemblance to *Thraustotheca primoachlya*. In the present species, sporangia are produced in great abundance; first, a type which resembles *Achlya*, to be followed later by a modified *Thraustotheca* type. *Achlya* type sporangia are frequently produced on the same hyphae as the *Thraustotheca* type; however, they may often appear without the *Thraustotheca* type sporangia. The *Achlya* type sporangia are most frequently produced at the tips of the threads, while the *Thraustotheca* type very commonly are found farther back (fig. 1).

The writer wishes to express her sincere appreciation to Dr. W. C. Coker and to Dr. J. N. Couch for direction and encouragement during the progress of this study. The writer is also very grateful to Miss Alma Whiffen and to Dr. Leland Shanor for assistance and suggestions at various times.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

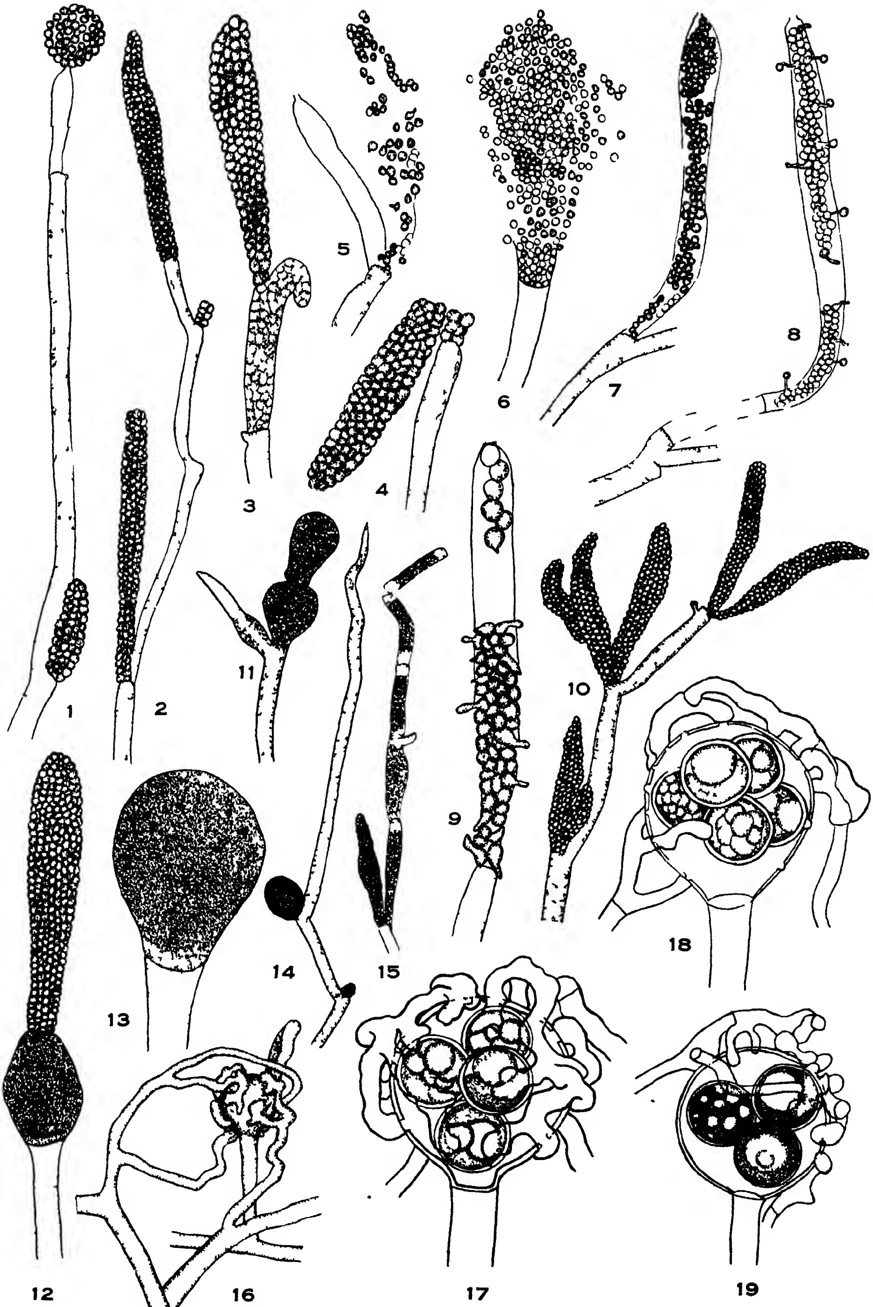
LITERATURE CITED

COKER, W. C.
1923 The Saprolegniaceae. Chapel Hill, North Carolina.
1927 Other Water Molds from the Soil. Jour. E. M. Sci. Soc. **42**: 207–226.
COKER, W. C., AND COUCH, J. N.
1923 A New Species of *Thraustotheca*. Jour. E. M. Sci. Soc. **39**: 112–115, pl. 8.
1924 Revision of the Genus *Thraustotheca*, with a Description of a New Species. Jour. E. M. Sci. Soc. **40**: 197–202, pls. 38–40.
COKER, W. C., AND HYMAN, O. W.
1912 *Thraustotheca clavata*. Mycologia **4**: 87–90.
COUCH, J. N.
1931 Observations on Some Species of Water Molds Connecting *Achlya* and *Dictyuchus*. Jour. E. M. Sci. Soc. **46**: 225–229.
1932 The Development of the Sexual Organs in *Leptolegnia caudata*. Amer. Jour. Bot. **19**: 584–597.
HARVEY, J. V.
1925 A Study of the Water Molds and Pythiums Occurring in the Soils of Chapel Hill. Jour. E. M. Sci. Soc. **41**: 151–164.
RAPER, J. R.
1936 Heterothallism and Sterility in *Achlya* and Observations on the Cytology of *Achlya bisexualis*. Jour. E. M. Sci. Soc. **52**: 274–293.
WESTON, WM. H.
1918 The Development of *Thraustotheca*, a Peculiar Water-Mould. Ann. Bot. **32**: 155–170.

EXPLANATION OF PLATE 31

Fig. 1. Tip of hypha showing both an *Achlya* and a *Thraustotheca* type of sporangium. × 462.
Fig. 2. End of hypha showing two *Thraustotheca* type sporangia and a stub of one. × 462.
Fig. 3. Hyphal tip showing a *Thraustotheca* type sporangium and under it a younger developing sporangium. × 88.
Fig. 4. Hyphal tip showing part of sporangium breaking away. × 88.
Fig. 5. End of hypha showing sporangium which has already discharged spores as in *Achlya* and a *Thraustotheca* type sporangium from which most of the spores have escaped. × 62.
Fig. 6. Hyphal tip showing a sporangium of the *Thraustotheca* type in which the spore mass has crumbled apart. × 88.
Fig. 7. Portion of hypha showing a sporangium of the *Achlya* type from which part of the spores have escaped, the remainder encysted within the sporangium. × 68.
Fig. 8. A similar sporangium in which some of the spores are sprouting through the sporangium wall and liberating the second swimming stage. × 50.
Fig. 9. A peculiar sporangium with the upper part of *Achlya* type, the lower part *Thraustotheca* type. × 115.
Fig. 10. Hyphal tip showing characteristic clusters of sporangia. × 22.
Fig. 11. End of hypha showing two gemmae which have formed at the tip, with the continuation of growth from below. × 208.
Fig. 12. Hyphal tip bearing a *Thraustotheca* type sporangium at the end and a fusiform gemma below. × 50.
Fig. 13. Hyphal tip bearing a terminal clavate gemma. × 155.
Fig. 14. End of hypha showing an oval gemma at the side and below it a few spores that remained in place after the rest of the sporangium had broken away. × 22.
Fig. 15. Cylindrical gemmae formed by the segmentation of a hypha, the end one breaking away. × 22.
Fig. 16. Habit sketch of young sporangium with diclinous antheridia. × 50.
Fig. 17. Oogonium with branched antheridia, pits in the oogonial wall, and eggs in different stages of maturation. × 208.
Fig. 18. Combined median and surface view of oogonium showing numerous pits. Eggs in various stages of development. × 208.
Fig. 19. Smaller unpitted oogonium with smaller eggs. × 252.

PLATE 31



OBSERVATIONS ON RHIZOPHLYCTIS ROSEA*

BY MARY WILLIAMS WARD

PLATES 32 AND 33

The fungus *Rhizophlyctis rosea* was discovered in 1862 growing saprophytically on damp rich soil in flower pots, producing here and there a rose color, and was originally described by De Bary and Woronine (1864; reprinted 1865) as *Chytridium roseum.* Cornu (1869) reported it a second time growing apparently as a parasite on the spores of *Equisetum arvense* which had been kept under cover at high humidity. It was found a third time by Sorauer in Germany on damp blotting paper (Schröter, 1889). Fischer transferred *Chytridium roseum* to the genus *Rhizophlyctis.* Fitzpatrick (1930) includes the genus *Rhizophlyctis* in the Rhizidiaceae, but states that all the species in this genus occur on algae.

Until recently (Couch, 1939) this fungus has been reported only from Europe and in spite of its wide distribution (see below) is very inadequately known. Recently several collections have been obtained in our laboratory. Dr. J. N. Couch first isolated it in November, 1938, from a soil sample taken in the vicinity of Chapel Hill. Miss Alma Whiffen has isolated it from soil collections made near Hammonton, New Jersey. Mr. Hiden Cox has obtained it from soil samples sent to Dr. R. E. Coker from the Galapagos Islands and also from water collections from near Florence, South Carolina. The present author has isolated it from water collections made in the vicinity of Burgaw, North Carolina. This species has been quite easy to culture and because of this fact it has been possible to work out most of the details in the life history.

The following description, with the exception of that of resting spores, has been made from material growing on corn leaves in distilled water.

Rhizophlyctis rosea (DeBary and Woronine) Fischer

Thallus monocentric. Each thallus consisting at maturity either of a zoosporangium with an extensively developed rhizoidal system, or of a

* Material in this paper taken from a thesis presented in partial fulfillment of the requirements for the master's degree in the Department of Botany, University of North Carolina.

resting body with rhizoids. Zoosporangia rose-colored, smooth-walled, spherical or irregular in shape, quite variable in size, up to 130 μ wide. Exit papillae 1–several, of varying length and diameter, the ends being filled with a gelatinous plug; rhizoids arising from 1–several places on the zoosporangium, stout, up to 11.66 μ in thickness near the point of origin, extensive, much branched, sometimes 650 μ or more in length; zoospores emerging through the papillae, about spherical, mostly 4 μ thick, with a small refractive globule and a very long posterior cilium. Resting spores smooth, relatively thick-walled, spherical, oval or irregular, contents olive-brown to orange-brown in color, granular and containing numerous refractive bodies. Germination not observed. Saprophytic or weakly parasitic (according to Cornu).

HOST RANGE AND CULTURE EXPERIMENTS

This plant was isolated on corn and grass leaves which contained chlorophyll and on corn leaves from which the chlorophyll had been extracted. This was done by cutting out a small section of the leaf infected by the fungus, that had previously been thoroughly washed, then transferring it to a sterile Petri dish to which a small amount of water and two or three small pieces of cooked grass were added. Monosporangial cultures were obtained by cutting out a single sporangium from an infected leaf and by transferring it to a sterile Petri dish containing a piece of cooked grass and a small amount of water. The most successful technique employed for obtaining cultures that were practically free from bacteria was the single spore method involving the use of agar (Couch, 1939). In using this method a single sporangium that was almost ready to discharge spores was cut out, washed several times, and placed on a sterile slide until the spores were discharged. By means of a capillary pipette, a drop of water containing spores was drawn up and blown out on a plate of agar. By tilting the plate several times the spores were widely distributed in such a manner that some were free from bacteria. After eight hours' growth, blocks of agar containing single growing plants were cut out and transferred to other plates of agar or to sterile Petri dishes containing a single piece of corn leaf.

In using the agar method, agars of weak concentration are necessary. Excellent results were obtained with both cornmeal and cellulose agar (for method, see Waksman, 1927, p. 197), but no satisfactory results were obtained with maltose-peptone #5 or with grass decoction agar. Growth, as a rule, is more rapid and maturity is reached more quickly on cornmeal than on cellulose agar. On cornmeal agar, sporangia often reached their mature size and discharged their spores after forty-eight hours, while on cellulose agar no sporangia discharged their spores when less than six days old without the addition of water. The spo-

rangia produced on cellulose agar are much more varied in shape than those produced on cornmeal agar, often being pyriform, while on cornmeal they are spherical in practically every instance. A few sporangia grown on cellulose agar attained a diameter of 123.28 μ after seven days' growth. A comparison of the average size of sporangia observed at regular intervals when growing on cornmeal agar is shown in the table below:

CULTURE MEDIA	PERIOD OF GROWTH	AVERAGE DIAMETER OF SPORANGIA
Cornmeal agar	23 hours	13.28μ
Cellulose agar	23 hours	5.33μ
Cornmeal agar	48 hours	21.65μ
Cellulose agar	48 hours	16.66μ
Cornmeal agar	60 hours	53.57μ
Cellulose agar	60 hours	21.65μ
Cornmeal agar	7 days	71.63μ
Cellulose agar	7 days	83.30μ

Cultures were carried on almost exclusively on pieces of corn leaves, thriving equally well on leaves containing chlorophyll and on ones from which the chlorophyll had been removed.

Experiments were tried to determine whether or not this species would live on other substrata. The results are recorded in the table below:

SUBSTRATUM	RESULTS
1. *Equisetum* spores	Some sporangia
2. *Lycopodium* spores	No sporangia
3. Peach petals (boiled)	No sporangia
4. Cherry petals (boiled)	No sporangia
5. Japonica petals (boiled)	Few sporangia
6. Japonica petals (frozen)	Few sporangia
7. Rose petals (boiled)	No sporangia
8. Spirea petals (boiled)	No sporangia
9. Violet petals (boiled)	Few sporangia
10. Viburnum petals (boiled)	Few sporangia
11. Corn leaves (uncooked)	Many sporangia
12. Spider lily leaves (chlorophyll extracted)	Many sporangia
13. Elodea leaves (chlorophyll present)	Some sporangia
14. Elodea leaves (chlorophyll extracted)	Some sporangia
15. Pine pollen	No sporangia
16. Sweet gum pollen	No sporangia
17. Filter paper	Few sporangia
18. Chitin (boiled slices of cow horn)	No sporangia

DEVELOPMENT AND STRUCTURE OF THE THALLUS

As De Bary and Woronine (1864) and Cornu (1869) have correctly shown, the zoospores emerge fully formed. However, De Bary and Woronine state that they remain motionless for an instant after being discharged before commencing the jerky movements. I have observed that in a few cases some of the spores may remain motionless for an instant or so, but they usually begin the characteristic jerky movement immediately upon emergence. They may dart forward with great rapidity, come to an abrupt stop, and then dart off again. The flagellum is in a posterior position when the spores are swimming. If the zoospores are obstructed or get caught or trapped in a position in which it is impossible for them to swim about, they may become somewhat amoeboid for a short time (Pl. 32, figs. 1–5), then round up and dart off again as soon as becoming free. The length of the active swimming period, according to my observations, varies from approximately twenty minutes to three hours. However, when a few zoospores become trapped in a sporangium they have been observed in some cases to remain active for as long as ten hours. The zoospores are hyaline, generally spherical (Pl. 32, figs. 6–7), about 4μ in diameter, with a single posteriorly attached flagellum which is approximately five times as long as the diameter of the zoospore. The most conspicuous structure in the living zoospore is a round, highly refractive globule. After the zoospore comes to rest on the substratum it loses its cilium and begins to enlarge (Pl. 32, figs. 8–9). A membrane develops and the single refractive body seen in the swimming spore breaks up into several smaller refractive bodies (Pl. 32, figs. 9–10). Along with these changes the rhizoids begin development (fig. 11). The central portion of this developing thallus continues to grow and forms a sporangium.

The refractive globules of the developing thallus increase in number as the sporangium grows and have a tendency to collect in an eccentric position (figs. 13–14). As the globules increase in size, they assume a rose tint which becomes more and more evident, until by the time the zoosporangium has reached the stage shown in fig. 14, the globules are deeply rose-colored and highly refractive. As the sporangium matures the large globules are broken up into smaller ones which become evenly dispersed through the alveolate cytoplasm. Those globules now seem to be digested until a condition is reached in which the cytoplasm is filled with tiny globules (fig. 16). These globules now collect into small groups, the globules in each group coalescing to form the conspicuous

globule of the spore. At this stage, the protoplasm assumes a dull rose tint.

In the maturation of the sporangium from the stage shown in fig. 14, from one to several papillae, filled with very condensed hyaline protoplasm that is demonstrable by staining with gentian violet, are formed as outgrowths of the sporangial wall.

Simultaneously with the development of the sporangium, an elaborate rhizoidal system consisting of several main trunks from which arise much-branched delicate threads is formed (fig. 25). The threads, however, do not anastomose or taper at the ends, becoming as mere lines, but retain always a tubular appearance with blunt ends. In the early stages of sporangial development the protoplasm in the rhizoids appears quite hyaline, but its presence can be demonstrated by killing material in the fumes of osmic acid and staining with gentian violet. By the time the sporangium has reached its mature size, the rhizoids are empty and are separated from the sporangium by cross-walls (fig. 15, etc.).

Cleavage of the protoplasm into spores may begin within twenty-four hours after the granular stage is reached, depending upon the condition of the culture. Figure 17 shows a sporangium in which the process of cleavage is complete and the individual zoospores have been delimited. At this stage the pigment disappears. Due to the mutual pressure of the zoospores on each other, they are angular in shape.

As the spores are being formed the hyaline plug in the papilla is gradually pushed out to the end and finally becomes rounded (fig.17).

The immediate indication that the spores are going to emerge is the extrusion of the hyaline plug from the end of the papilla (fig. 17). After the spores have milled around in the sporangium for a few seconds, they emerge through the exit papillae as rapidly as possible (fig. 18). The length of time required for the emergence of all of the spores is dependent to a large degree upon the size of the sporangium and whether or not some of the spores become trapped inside.

The shortest time observed from spore germination to the maturity of the sporangium and the discharge of the spores was forty-eight hours when grown on cornmeal agar. The average time elapsing between spore germination and the maturity of the sporangia on corn leaves is approximately three days. Spores are discharged at the end of this period or from several days to a week or two later, depending upon the condition of the culture.

The mature zoosporangia of *Rhizophlyctis rosea* are highly variable

in size and shape. They may be spherical, oval, elliptical, clavate, cubical, conical, or irregular with from one to ten, or perhaps more, exit papillae which may vary in length and in diameter (figs. 19–23).

The size of the zoosporangia range from approximately 4μ to 130μ in diameter. The number of zoospores produced in a single sporangium varies, depending upon its size.

The walls of the sporangia and of the papillae give a pale purplish or lavender color with chloriodide of zinc, thus indicating a cellulose composition. The rhizoids at the same time remain perfectly colorless.

Immature resting bodies of a *Rozella* (?) have been observed a few times parasitizing sporangia of this fungus (fig. 31).

Resting spores as well as zoosporangia of *Rhizophlyctis rosea* were found by Prof. Couch on a piece of cardboard collected by him March 8, 1939, at the Caves near Chapel Hill, and the observations below were made from these. Mature resting spores are smaller than sporangia and less variable in size and shape (figs. 26–30). Their walls are smooth and relatively thick, with contents varying in color from olive-brown to orange-brown. Numerous highly refractive fat globules are present. Neither the development nor the germination of the resting spores has been observed.

DISCUSSION

The present fungus may be easily distinguished from *Rhizophlyctis Petersenii* as described by Sparrow (1937). In *Rhizophlyctis Petersenii* the protoplasm of the growing plants contains numerous orange-brown bodies and only one exit papilla is produced. In *Rhizophlyctis rosea* the protoplasm in the growing plant is rose-colored and one or many exit papillae may be present. In *Rhizophlyctis Petersenii* the spores emerge in a mass and form a large, spherical, possibly hollow, motionless cluster at the orifice of the tube, while in the present fungus the spores emerge singly, fully formed, and begin their characteristic jerky movement immediately. Another conspicuous difference exhibited by the two species, however, is the possession of a small orange-brown globule in the spores of *Rhizophlyctis Petersenii* in contrast to the absence of a pigment spot in the spores of *Rhizophlyctis rosea.*

SUMMARY

1. A Chytrid, *Rhizophlyctis rosea,* previously incompletely described, has been isolated in single spore culture and has been grown on various substrata.

2. The development and structure of *Rhizophlyctis rosea* has been described.

3. Resting bodies which are varied in size and shape occur in *Rhizophlyctis rosea.* Neither their development nor their germination has been observed.

ACKNOWLEDGEMENTS

The writer wishes to express sincere appreciation to Dr. J. N. Couch and to Dr. W. C. Coker for direction and encouragement during the progress of this study. The writer is also very grateful to Miss Alma Whiffen and to Dr. Leland Shanor for assistance and suggestions at various times.

DEPARTMENT OF BOTANY,
THE UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

LITERATURE CITED

CORNU, MAX.
1869 Note sur le *Chytridium roseum.* Bull. Soc. Bot. de France **16**: 223–224.

COUCH, J. N.
1939 Technic for Collection, Isolation, and Culture of Chytrids. Jour. E. M. Sci. Soc. **55**: 208–214.

DEBARY AND WORONINE.
1864 Berichte über d. Verhandl. d. naturf. Ges. zu Freiburg **3**, Heft 2, p. 54, figs. 17–20.
1865 Supplément a l'Histoire des Chytridinées. Ann. Sci. Nat., ser. 5, **3**: 239–269, Pl. 10, figs. 17–20.

FISCHER, A.
1892 Archimycetes (Chytridinae) in Rabenhorst's Kryptogamen-Flora I, **4**: 3–160.

FITZPATRICK, H. M.
1930 The Lower Phycomycetes. McGraw-Hill Book Company: New York.

SCHROETER, J.
1889 Kryptogamen-Flora von Schlesien **3**, Pt. 1: 191.

SPARROW, F. K., JR.
1937 Some Chytridiaceous Inhabitants of Submerged Insect Exuviae. Proc. Am. Phil. Soc. **78**: 23–53.

WAKSMAN, S. A.
1927 Principles of Soil Microbiology. The Williams & Wilkins Company. Baltimore.

EXPLANATION OF PLATES

PLATE 32

Figs. 1–5. Living zoospores. × 472.

Figs. 6–7. Zoospores killed in the fumes of osmic acid and stained with gentian violet. × 600.

Fig. 8. Spore which has settled down and lost its cilium. × 250.

Fig. 9. Enlargement of spore and breaking up of single refractive body into several smaller ones. × 250.

Figs. 10–13. Early stages in spore germination and development of sporangium on cellulose agar. Spores are not growing in contact with bacteria. × 250.

Figs. 14–18. Stages in development of sporangium, spores, and spore discharge in the same sporangium. × 208.

Figs. 19–23. Variations in the shapes and sizes of sporangia. × 208.

Fig. 24. Characteristic cluster of sporangia. × 68.

Fig. 25. Habit sketch of thallus showing about one fourth of the rhizoids. × 88.

Figs. 26–30. Variations in the shape and size of resting spores. × 208.

Fig. 31. Immature resting body of a species of *Rozella* (?) parasitic on a sporangium of *Rhizophlyctis rosea* × 208.

PLATE 33

Photomicrographs

Fig. 1. Portion of corn leaf showing infection. × 50.

Fig. 2. Mature zoosporangium showing extensive rhizoidal system. × 187.

Fig. 3. Single immature zoosporangium showing extensive rhizoidal system. × 120.

Fig. 4. Characteristic cluster of sporangia growing on corn leaf. × 80

Fig. 5. Single zoosporangium in which the zoospores are ready to be discharged. One hyaline plug which has been extruded is shown at the end of the papilla. × 240.

Fig. 6. Single zoosporangium almost ready to discharge spores. Hyaline plugs are shown on the tips of the papillae. × 317.

Fig. 7. Single zoosporangium which has been growing on cornmeal agar 30 hours free from bacteria. Bacteria are shown at the upper edge of the picture × 417.

Fig. 8. Resting spore. × 50.

Fig. 9. Resting spore. × 50.

PLATE 32

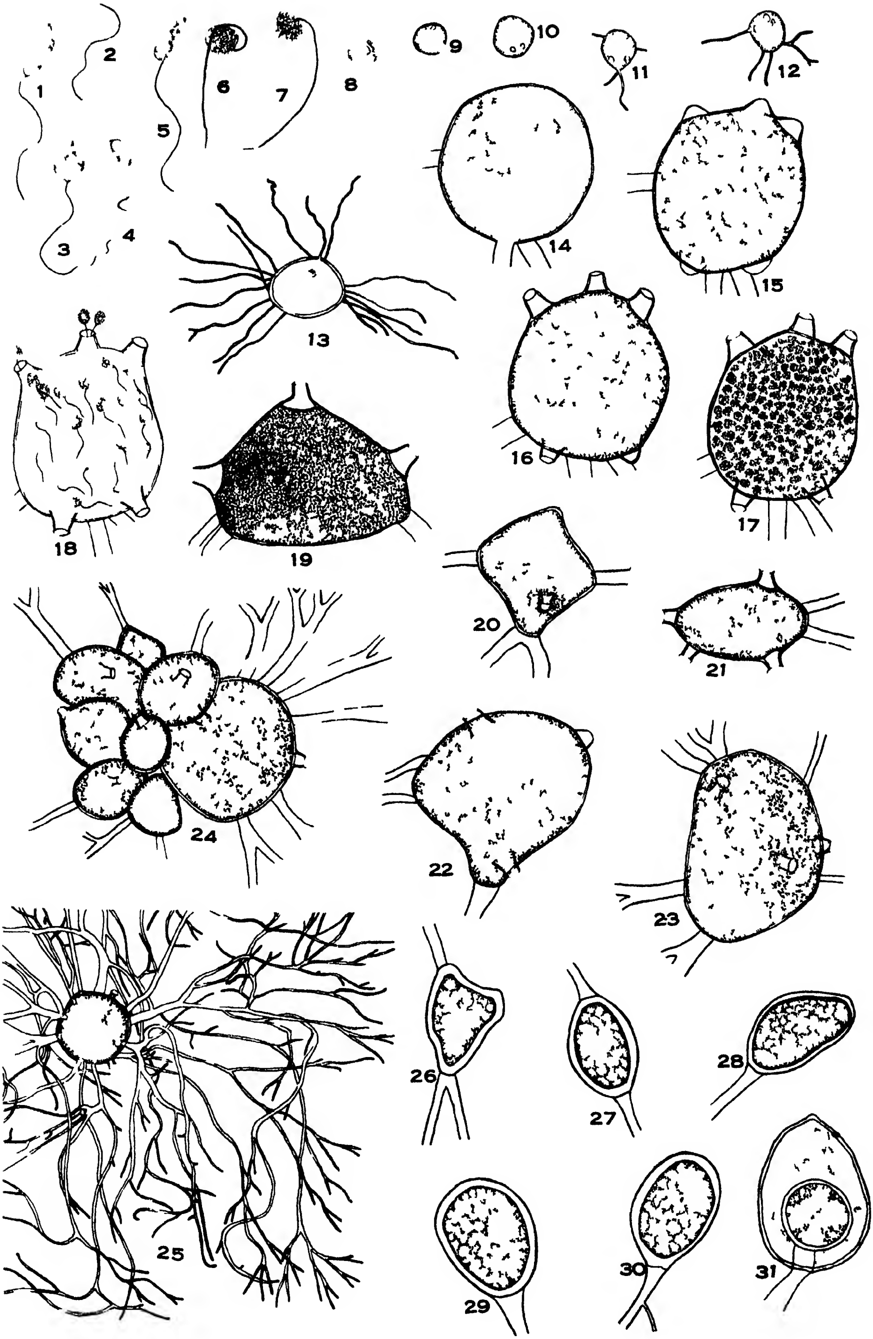
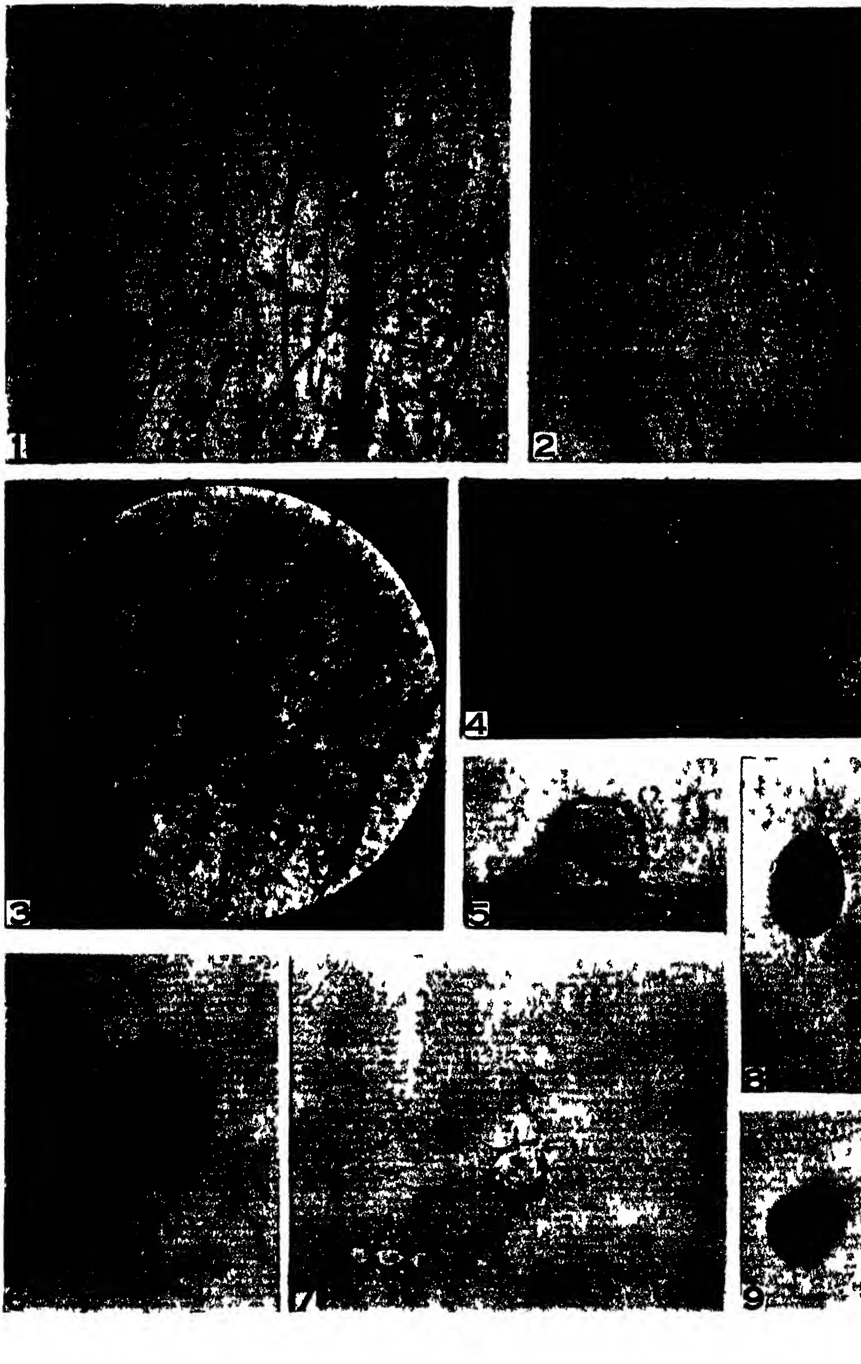

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31

PLATE 33



SOME FLORIDA GILL-FUNGI

By William A. Murrill

In a recent article in this journal the author discussed species of the genus *Agaricus* found in the vicinity of Gainesville, Fla., including two described as new. The present paper includes novelties from the same region in several other genera of the gill-fungi, notably in *Cortinarius*. This difficult genus was studied for many years by Dr. C. H. Kauffman, who listed 202 species for North America in his treatment in "North American Flora" published in 1932.

The genus *Cortinarius* is peculiarly northern in its range. Names of southern states are rare in Dr. Kauffman's list, and only two tropical American localities are known, both in the mountains. Species now found in the Oligocene Island area about Gainesville must have migrated there during or following the successive glacial epochs of the Pleistocene; or else originated in the region through variation and mutation.

The commonest species in this area is *C. lilacinus* Peck; and *C. semisanguineus* (Fr.) C. H. K. is not rare, while its near relative, *C. cinnamomeus* (L.) Fr., is less frequent. An interesting case of migration and adaptation is found in *C. camphoratus* Fr. Kauffman lists it only from the Adirondacks in this country, the type locality being Sweden. I have found it here in four different places, always with its characteristic odor. One would judge that it reached its new southern home at a later date than *C. lilacinus*. Unquestionably many more migrating species of the genus perished than survived, or during their period of adaptation became something quite different.

Most of the species here described as new show a close relationship to some northern species, just as one would expect, but they have not retained their fondness for cool weather as some other migrating gill-fungi have done. This, I think, is due largely to the fact that they are very fleshy and must have plenty of water, which is usually impossible during a Florida winter. Given, then, the demand for water, which is met by the summer rains, and the radical change from a cool to a hot climate, and we have the physical basis for the origin of endemic species in the Oligocene Island region.

The collections here cited by numbers are all deposited permanently

in the herbarium of the Florida Agricultural Experiment Station, at Gainesville, where Mr. Erdman West is Mycologist. He has been for many years an enthusiastic collector and student of the fungi of Florida.

KEY TO ALACHUA CORTINARIUS

A. *Myxacium.* Pileus and stipe slimy-viscid.	
Pileus bay; stipe lilac	1. *C. Arnoldae*
B. *Bulbopodium.* Pileus viscid; stipe dry, bulb marginate.	
Pileus pallid to isabelline; stipe very short	2. *C. praebrevipes*
Pileus violet-white; spores 11–12 × 5–6 μ	3. *C. subglaucopus*
Pileus sulfur-colored with testaceous center	4. *C. subfulmineus*
C. *Phlegmacium.* Pileus viscid; stipe dry, clavate.	
Pileus white with rosy-isabelline tint; spores very warty	5. *C. subcommunis*
Pileus violet-isabelline; spores uneven, 15 μ long	6. *C. sublargus*
Pileus isabelline to fulvous; spores smooth, 7–8 μ long	7. *C. subcaespitosus*
Pileus avellaneous; spores subglobose	8. *C. praefelleus*
Pileus avellaneous; spores ovoid	9. *C. Westii*
D. *Inoloma.* Pileus dry; stipe stout, clavate.	
Pileus white; spores warty	10. *C. albidulus*
Pileus lilac; spores smooth	11. *C. lilacinus*
Pileus lavender; spores rough; odor fetid	12. *C. camphoratus*
Pileus pale-avellaneous; spores warty	13. *C. subargentatus*
Pileus ferruginous; spores smooth	14. *C. perferrugineus*
E. *Dermocybe.* Pileus dry; stipe slender.	
Pileus isabelline, tomentose	15. *C. subflavifolius*
Pileus fulvous; stipe bluish	16. *C. hiemalis*
Pileus yellowish-cinnamon; stipe yellow; gills yellow	17. *C. cinnamomeus*
Pileus cinnamon-yellow; stipe yellow; gills blood-red	18. *C. semisanguineus*
F. *Hydrocybe.* Pileus hygrophanous, without annulus.	
Pileus isabelline with fulvous disk	19. *C. subjuberinus*

Cortinarius albidulus sp. nov.

Pileo convexo-subexpanso, 4–5 cm. lato, albido; lamellis adnexis, latis, sporis ovoideis, 8 x 6 μ; stipite pallide violaceo, bulboso, 4 x 1 cm.

Pileus convex to subexpanded, gregarious, 4–5 cm. broad; surface smooth, glabrous, white or stramineous, margin even, entire, at first inflexed; context pallid, odorless, mawkish but not very disagreeable; lamellae adnexed, rounded behind, ventricose, broad, crowded, entire, ferruginous-fulvous when seen; spores ovoid, often inequilateral, obliquely apiculate, deep-ferruginous, conspicuously tuberculate, usually 1-guttulate, about 8 x 6 μ; cystidia none; stipe equal above the large rounded bulb, smooth, glabrous, pale-violet, about 4 x 1 cm.

Type collected by W. A. Murrill under a live-oak in Gainesville, Fla., Jan. 13, 1938 (*F15879*). Much resembling *C. lilacinus* in shape but differing in color, taste, and spore characters. Like *C. hiemalis*, it seems fond of cool weather.

Cortinarius hiemalis sp. nov.

Pileo convexo-subexpanso, 5–7 cm. lato, fulvo; lamellis sinuatis, sporis ovoideis, 8–10 x 5–6 μ; stipite lilacino-albo, 5 x 0.6–0.8 cm.

Pileus convex to subexpanded, slightly umbonate at times, gregarious, 5–7 cm. broad; surface dry, smooth, glabrous, subshining, uniformly fulvous, margin even, entire to undulate; context thin, white, unchanging, odorless, mild, nutty; lamellae sinuate, inserted, ventricose, medium broad, medium distant, entire, fulvous when seen; spores ovoid, apiculate, granular, finely tuberculate, subfulvous, 8–10 x 5–6 μ; cystidia none; stipe equal or slightly enlarged below, smooth, glabrous, satiny-white with a faint bluish tint, solid, 5 x 0.6–0.8 cm.; cortina slight, evanescent.

Type collected by W. A. Murrill on the ground in an oak-pine grove in Gainesville, Fla., Jan. 10, 1938 (*F 15951*). A trim, neat species with fulvous cap and glaucous stem, appearing only in winter.

Cortinarius perferrugineus sp. nov.

Pileo convexo-expanso, umbonato, ferrugineo, 5–7 cm. lato, fibrilloso; lamellis sinuatis, sporis glabris, 12 x 5–6 μ; stipite fibrilloso, bulboso, 4–6 x 1.5–2 cm.

Pileus convex to expanded, broadly umbonate, ferruginous throughout, gregarious, 5–7 cm. broad; surface dry, innate-fibrillose-tomentose, margin even, entire; context thick, isabelline, unchanging, sweet, odorless; lamellae sinuate, inserted, ventricose, rather broad, subdistant, entire; spores elongate-ellipsoid, obliquely apiculate, smooth, 1-guttulate, pale-ferruginous, about 12 x 5–6 μ; cystidia none; stipe fibrillose, solid, 4–6 x 1.5–2 cm., the ovoid bulb 3 cm.; veil fibrillose, evanescent.

Type collected by W. A. Murrill on the ground in oak-pine woods in Gainesville, Fla., July 15, 1938 (*F 18417*). A monochrome species not rare in woods about Gainesville.

Cortinarius praefelleus sp. nov.

Pileo convexo-expanso, 6.5 cm. lato, praeviscido, avellaneo, felleo; lamellis adnatis, confertis, umbrinis, sporis subglobosis, 6–8 μ longis; stipite caesio, bulboso, 3 x 1.4 cm.

Pileus convex to expanded, gregarious, 6.5 cm. broad; surface soft, slimy-viscid, glabrous, uneven, avellaneous with cremeous areas, margin entire, even; context thick, whitish, unchanging, fragrant, soon very bitter; lamellae adnate, inserted, close, medium broad, entire, umbrinous when seen; spores subglobose to broadly ellipsoid, densely and conspicuously tuberculate, deep-ferruginous, 6–8 μ long; cystidia none; stipe equal above the large subglobose bulb, smooth, caesious, fibrillose, 3 x 1.4 cm., bulb 2 cm. thick and 1.5 cm. high.

Type collected by E. West and W. A. Murrill under hardwood trees in Planera Hammock, eleven miles northwest of Gainesville, July 20, 1938 (*F 18414*). Also collected by West, Arnold, and Murrill in Sugarfoot Hammock, July 11, 1938 (*F 17799*). Distinctive by reason of the shape and color of its spores, which are usually rounded, rough, and dark, the dried gills being dull-umbrinous. The very bitter flesh is another good character, not to mention its fragrance. All in all, a decidedly interesting species, suggesting *C. infractus* Fries.

Cortinarius subargentatus sp. nov.

Pileo convexo-plano, 6 cm. lato, pallide avellaneo, glabro; lamellis adnatis, sporis tuberculatis, 10 x 5 μ; stipite albido, bulboso, 4 x 1–2 cm.

Pileus convex to plane, gregarious, 6 cm. broad; surface dry, smooth, glabrous, shining, pale-whitish-avellaneous, margin even, entire, slightly upturned with age; context thin, whitish, sweet, odorless; lamellae adnate, inserted, medium distant, medium broad, entire, fulvous when seen; spores ellipsoid, tuberculate, 1-guttulate, deep-ferruginous, about 10 x 5 μ; cystidia none; stipe smooth, subglabrous, bulbous, shining, white with a pale-violet tint, 4 x 1–2 cm.

Type collected by West, Arnold, and Murrill under hardwood trees in Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 21, 1938 (*F 18409*). A very attractive species suggesting *C. lilacinus* Peck but having tuberculate spores. The bulb is shaped like a young onion, without any trace of a margin.

Cortinarius subcaespitosus sp. nov.

Pileo convexo-subdepresso, 6–10 cm. lato, isabellino ad fulvo, viscido; lamellis sinuatis, pallidis, sporis 7–8 x 4 μ; stipite albido, clavato, 3.5–4 x 1.5–3 cm.

Pileus convex to expanded, slightly depressed, cespitose, 6–10 cm. broad; surface smooth, glabrous, shining, viscid, isabelline to fulvous; margin paler, even, entire, inflexed when young, straight or slightly upturned with age; context very thick, 1 cm. or more, white, unchanging, odorless, mild but mawkish; lamellae sinuate or adnexed, rounded behind, ventricose, broad, close, inserted, entire to undulate, pallid with a violet tint, becoming dull-fulvous; spores almond-shaped, often inequilateral, smooth, 1-guttulate, deep-ferruginous, about 7–8 x 4 μ; cystidia none; stipe tapering upward, smooth, glabrous, shining, white with a violet tint, solid, clavate, not marginate, 3.5–4 x 1.5–3 cm.

Type collected by W. A. Murrill under hardwood trees in South Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 30,

1938 (*F 18413*). Cotype collected by the author under oaks at the same time and place (*F 18415*). Few members of this genus are cespitose. In the type three hymenophores were grown together at the base. It may occasionally be found solitary.

Cortinarius subflavifolius sp. nov.

Pileo convexo-subexpanso, 6 cm. lato, tomentoso, isabellino; lamellis adnatis, sporis ellipsoideis, tuberculatis, 6–8 x 5 μ; stipite non clavato, 4 x 1–1.5 cm.

Pileus convex to subexpanded, gregarious, 6 cm. broad; surface dry, tomentose, isabelline, margin even, entire; context hygrophanous, isabelline, odorless, mild; lamellae adnate, ventricose, rather broad, medium distant, inserted, entire, ferruginous; spores ellipsoid, granular, apiculate, conspicuously tuberculate, bright-ferruginous, fulvous in mass, about 6–8 x 5 μ; cystidia none; stipe subequal, subglabrous, 4 x 1–1.5 cm.; cortina fibrillose, evanescent, leaving no ring or ring-trace.

Type collected by West, Arnold, and Murrill under oaks in Sugarfoot Hammock, near Gainesville, Fla., July 12, 1938 (*F18412*). A smaller species than *C. flavifolius* Peck, with shorter stem and no trace of a ring. The spores, however, are much the same, varying from ellipsoid to almost subglobose.

Cortinarius subfulmineus sp. nov.

Pileo convexo-plano, 5–6 cm. lato, viscido, sulphureo testaceoque; lamellis sulphureis, sporis tuberculatis, 11–12 x 5–6 μ; stipite albido, bulboso, 5–7 x 0.7–1.3 cm.

Pileus convex to plane, gregarious to subcespitose, 5–6 cm. broad; surface viscid, smooth, glabrous, sulphureous, testaceous in the center, margin even, entire; context 1.5 cm. thick at the center, whitish, unchanging, with a strong mushroom odor and a mild, slightly mawkish taste; lamellae slightly uncinate, 5 mm. broad, plane, inserted, crowded, undulate on the edges, sulphur-colored; spores almond-shaped, 1-guttulate, deep-ferruginous, distinctly tuberculose, 11–12 x 5–6 μ; cystidia none; stipe tapering upward, smooth, glabrous, white with a sulphur tint, solid, white within, 5–7 x 0.7–1.3 cm.; bulb plainly marginate, 2.5 cm. thick and 1 cm. high; cortina evanescent except for a few fibrils on the stipe.

Type collected by West and Murrill under hardwood trees in South Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 26, 1938 (*F 18398*). An attractively colored species with large, margined bulb and tuberculate spores. Found only once.

Cortinarius subglaucopus sp. nov.

Pileo convexo-subexpanso, 3–5 cm. lato, viscido, violaceo-albo; lamellis adnexis, sporis ellipsoideis, tuberculatis, 7–8 x 5 μ; stipite concolori, 4–5 x 0.7–1.5 cm., bulbo marginato.

Pileus convex to subexpanded, gregarious, 3–5 cm. broad; surface somewhat viscid, smooth, glabrous, violet-white, becoming darker, margin even, entire; context thin, pallid, mild, odorless; lamellae adnexed, ventricose, rather narrow, crowded, entire, becoming fulvous; spores ellipsoid, 1-guttulate, distinctly tuberculate, deep-ferruginous, about 7–8 x 5 μ; cystidia none; stipe tapering upward, smooth, glabrous, shining, pale-violet-white, 4–5 x 0.7–1.5 cm., the bulb decidedly marginate.

Type collected by W. A. Murrill under hardwood trees in South Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 30, 1938 (*F 18407*). Resembling *C. alboviolaceus* but with marginate bulb and tuberculate spores.

Cortinarius subjuberinus sp. nov.

Pileo convexo-plano, 3–4 cm. lato, isabellino, disco fulvo; sporis ellipsoideis, 7–8 x 5 μ, stipite lilacino, clavato, 4 x 0.5 cm.

Pileus convex to plane, gregarious, 3–4 cm. broad; surface smooth, glabrous, hygrophanous, isabelline, fulvous in the center, margin even, entire; context thin, pallid, mild; lamellae adnate, broad, inserted, medium distant, undulate on the edges, fulvous, fragile with age; spores ellipsoid, sometimes inequilateral, 1-guttulate, tuberculate, deep-ferruginous, about 7–8 x 5 μ; cystidia none; stipe tapering upward from a clavate base, smooth, lilac-tinted, about 4 x 0.5 cm.; bulb small, immarginate; cortina soon vanishing, leaving a few fibrils on the stipe.

Type collected by E. West and W. A. Murrill under hardwood trees in South Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 26, 1938 (*F 18408*). A small, slender species with pale-lilac stem and immarginate bulb.

Cortinarius sublargus sp. nov.

Pileo convexo-plano, 5 cm. lato, praeviscido, violaceo-isabellino; lamellis adnatis, violaceis, sporis ellipsoideis, 15 x 6–8 μ; stipite albido, 5 x 1.3 cm.; annulo subfibrilloso, pallido.

Pileus convex to plane, solitary, 5 cm. broad; surface slimy-viscid, smooth, glabrous, isabelline with a violet tint, margin even, entire; context thin, white, odorless; lamellae adnate, subdistant, medium broad, entire, violet to fulvous; spores ellipsoid, uneven, granular,

deep-ferruginous, about 15 x 6–8 μ; cystidia none; stipe equal or slightly clavate, smooth, glabrous, white with a violet tint, 5 x 1.3 cm.; annulus superior, persistent, submembranous with a fibrillose margin.

Type collected by W. A. Murrill under an oak in Gainesville, Fla., July 9, 1938 (*F 18416*). Also collected by E. West in oak woods at Gainesville, Oct. 7, 1928 (*F 15729*). The spores appear rougher than they really are because of their very granular contents. The species is rare in oak woods about Gainesville, appearing during warm weather.

Cortinarius Westii sp. nov.

Pileo convexo-plano, 5–8 cm. lato, viscido, avellaneo; lamellis subdecurrentibus, confertis, sporis ovoideis, 5–7 x 5 μ; stipite bulboso, pallido, 4–5 x 0.9 cm., annulo nullo.

Pileus convex to plane, gregarious, 5–8 cm. broad; surface viscid, smooth, glabrous, grayish-avellaneous, margin even, entire; context thick, white, unchanging, odorless, slightly mawkish and astringent; lamellae slightly decurrent, arcuate, inserted, crowded, narrow, entire, umbrinous when seen; spores broadly ovoid, distinctly tuberculate, 1-guttulate, deep-ferruginous, about 5–7 x 5 μ; cystidia none; stipe slightly bulbous, smooth, glabrous, violet-white, shining, about 4–5 x 0.9 cm.; cortina slight, leaving no annulus.

Type collected by E. West and W. A. Murrill under hardwood trees in South Planera Hammock, eleven miles northwest of Gainesville, Fla., Oct. 26, 1938 (*F 18418*). The spores are rather distinctive, being broadly ovoid and conspicuously tuberculate.

Gomphidius alachuanus sp. nov.

Pileo convexo-subdepresso, 4.5 cm. lato, glabro, avellaneo-isabellino; lamellis praelatis, pallidis, sporis 18–20 x 6 μ; stipite cremeo, 5 x 1 cm.

Pileus convex to slightly depressed, cespitose, 4.5 cm. broad; surface smooth, shining, glabrous, avellaneous-isabelline, vinose when dried, margin even, entire or undulate; context pallid, vinose when dried, odorless, mild to astringent, very thin except at the center; lamellae decurrent, very broad, distant, inserted, entire, yellowish to avellaneous, black when dried; spores elongate, vacuolate, pale-brownish under the microscope, about 18–20 x 6 μ; cystidia none; stipe tapering downward, distinctly grooved, cremeous, vinose when dried, about 5 x 1 cm.

Type collected by E. West and W. A. Murrill on the ground in oak-pine woods at Newnan's Lake, Florida, Nov. 15, 1938 (*F 18402*). Noteworthy because of its change from pale colors to vinose when dried.

It occurs rarely about Gainesville in mixed woods during December and January.

Entoloma substrictius sp. nov.

Pileo campanulato-expanso, umbonato, 3–4 cm. lato, avellaneo; lamellis sinuatis, dentatis, sporis angulatis, 11–13 x 6–8 μ; stipite albo vel avellaneo, 6–8 x 0.5–1 cm.

Pileus campanulate to broadly convex with a prominent conic umbo, gregarious, 3–4 cm. broad; surface dry, shining, smooth, glabrous, avellaneous, dark-avellaneous on the umbo; margin even, entire, incurved, much upturned and often splitting with age; context very thin, white, odorless, taste neither farinaceous nor bitter but mawkish and unpleasant; lamellae sinuate, broad, especially behind, medium distant, inserted, very uneven and toothed on the edges, pallid to dull-pinkish; spores very angular, apiculate, 1-guttulate, pink, 11–13 x 6–8 μ; cystidia none; stipe tapering upward, often compressed, smooth, shining, fibrillose to glabrous, white or avellaneous, 6–8 x 0.5–1 cm.

Type collected by E. West and W. A. Murrill in pine sawdust under bushes by a lake in Cary Memorial Forest, Alachua Co., Fla., Nov. 19, 1938 (*F 18397*). Suggesting *E. strictius* (Peck) Sacc. but with slenderer spores and thicker stem. The latter varies greatly in different hymenophores, in some being 5 mm. at the apex and 10 mm. at the base.

Nolanea subconica sp. nov.

Pileo convexo, umbonato, 1.5–2 cm. lato, avellaneo-isabellino; lamellis liberis, praelatis, sporis angulatis, 8 x 6 μ; stipite castaneo, 2.5–3.5 x 0.1–0.15 cm.

Pileus convex to broadly convex, papillate, gregarious, 1.5–2 cm. broad; surface glabrous, slightly striate, avellaneous-isabelline, dark-avellaneous on the umbo, margin entire; context membranous, white, somewhat nutty, odorless; lamellae free or slightly adnexed, very broad, inserted, medium close, undulate on the edges, pallid to pink; spores very angular, 1-2-guttulate, pink, about 8 x 6 μ; cystidia none; stipe equal, pruinose at the apex, glabrous below, whitish-mycelioid at the base, castaneous, 2.5–3.5 x 0.1–0.15 cm.

Type collected by E. West and W. A. Murrill under bushes by a lake in Cary Memorial Forest, Alachua Co., Fla., Nov. 19, 1938 (*F 18406*). Suggesting *N. conica* (Peck) Sacc. but with very broad gills and convex rather than conic in shape.

Lactaria caeruleitincta sp. nov.

Pileo infundibuliformi, 9 cm. lato, albo ad isabellino, acrido; lamellis adnexis, confertis, furcatis, albis; sporis ellipsoideis, tuberculatis, 8–9 x 5–6 μ; stipite albo, caeruleotincto, 4 x 1.7–2 cm.

Pileus deeply infundibuliform, solitary, 9 cm. broad; soft and yielding, smooth, glabrous, white to isabelline, margin even, entire; context white, watery, slowly becoming distinctly acrid, with a sickening odor while drying; lamellae adnexed, tapering behind, narrow, close, many forked below the middle, entire, white, pale-brownish where bruised; latex copious, whitish; spores broadly ellipsoid, hyaline, distinctly but not prominently tuberculate, about 8–9 x 5–6 μ; sterile marginal cells few, hyaline, clavate, pointed, about 25 x 6 μ; stipe slightly tapering downward, smooth, glabrous, milk-white tinged with pale-caeruleous and showing more blue after picking, 4 x 1.7–2 cm.

Type collected by E. West and W. A. Murrill in mixed woods at Newnan's Lake, Fla., Nov. 15, 1938 (*F 18390*). When first collected there was a blue ring near the base of the stipe and later more blue color was observed on the upper part of the stipe. The fresh cap was unusually soft to the touch, as in *Boletus pallidus*. The spores resemble those of *L. vellerea* rather than the smooth ones seen in *L. subvellerea*, but the gills are close as in the latter species. I have no word for the sickening odor developed in the electric drier.

Russula albidicremea sp. nov.

Pileo convexo-subdepresso, 6 cm. lato, albido, sapore grato; lamellis adnatis, distantibus, sporis flavidis, 8 x 6 μ; stipite albo, 4 x 1.5 cm.

Pileus convex to slightly depressed, solitary, 6 cm. broad; surface somewhat viscid, smooth, glabrous, opaque, whitish, yellowish at the center; margin deflexed, even, entire, peeling rather easily; context firm, rather thick, odorless, mild, white, unchanging; lamellae adnate, many forked at the base and many others inserted a few millimeters from the stipe, practically all equal, rarely forked near the tip, nearly plane, medium broad, subdistant to distant, entire, white to cremeous; spores ochroleucous in mass, broadly ellipsoid, distinctly but not prominently tuberculate, 1-guttulate, about 8 x 6 μ; cystidia none; stipe subequal, smooth, glabrous, milk-white, unchanging, solid, 4 x 1.5 cm.

Type collected by W. A. Murrill under oaks in woods at Gainesville, Fla., Nov. 17, 1938 (*F 18396*). Near *R. albiduliformis* Murrill but not striate nor milk-white and the gills are too distant. The name refers to the whitish surface and cremeous gills. The flesh is firm like that of *Tricholoma Russula*.

Lepiota truncatispora sp. nov.

Pileo convexo-expanso, umbonato, 1–1.5 cm. lato, hispido-tomentoso, fulvo, disco badio; sporis truncatis, 8 x 3–4 μ; stipite fibrilloso, 4 x 0.3 cm.

Pileus convex to expanded with a broad umbo, gregarious, 1–1.5 cm. broad; surface dry, finely hispid-tomentose, fulvous, bay on the umbo,

margin even, entire to undulate; context very thin, white, mild, the odor somewhat unpleasant; lamellae free, rounded behind, medium broad, crowded, inserted, white, unchanging, the edges conspicuously toothed; spores oblong, broad and obliquely truncate at one end, tapering at the other, smooth, hyaline, 1-guttulate, about 8 x 3–4 μ; cystidia none; stipe equal, fibrillose, white, hollow, about 4 x 0.3 cm.; annulus median, white, fixed, slight or evanescent.

Type collected by E. West and W. A. Murrill on the ground in mixed woods at Newnan's Lake, Fla., Nov. 15, 1938 (*F 18395*). The spores are peculiar and exceedingly abundant, varying only slightly in size or shape.

Cortinellus formosus sp. nov.

Pileo convexo-depresso, caespitoso, 5–8 cm. lato, testaceo, latericio- squamuloso, malodoro; lamellis albis, sporis 5–7 μ longis; stipite concolori squamulosoque, 6–8 x 1–2 cm.

Pileus convex to slightly depressed, densely cespitose, 5–8 cm. broad; surface dry, pale-testaceous, conspicuously and densely decorated with elongate, latericious scales upturned and setose at the tip; context thick, firm, pallid, with a strong, disagreeable, earthy odor and taste; lamellae sinuate, inserted, narrow, about 5 mm., crowded, white to dull-yellowish, beautifully fringed on the edges; spores subglobose to broadly ovoid, smooth, hyaline, finely granular, 5–7 μ long; sterile marginal cells fusoid, smooth, hyaline, abundant, cespitose, obtuse at the tapering tip, about 60–70 x 15–20 μ; stipe enlarged below, hollow, clothed and colored like the pileus, about 6–8 x 1–2 cm.

Type collected by Erdman West in pine sawdust by a pond in the Cary Memorial Forest, Alachua Co., Fla., Nov. 18, 1938 (*F 18405*). A striking and beautiful species with an unlovely odor (graveolens). The fringe on the gills is unusually fine.

Melanoleuca adusta sp. nov.

Pileo convexo-plano, 2–3 cm. lato, subumbrino ad isabellino, marginato; lamellis sinuatis, sporis ovoideis, 5 x 3 μ; stipite carnoso, rubro-brunneo, 3 x 0.2–0.3 cm.

Pileus convex to plane or umbilicate, gregarious, 2–3 cm. broad; surface pale-umbrinous when moist, isabelline when dry, subglabrous, smooth, margin even, entire, becoming reddish-brown or darker as though scorched; context thin, opaque-whitish, odorless, mild; lamellae sinuate, varying to adnexed, ventricose, medium broad, inserted, medium distant, undulate, pallid, scarcely changing when dried; spores ovoid, smooth, hyaline, 1-guttulate, about 5 x 3 μ; cystidia none; stipe tapering upward, fleshy, hollow, finely striate, glabrous, reddish-brown, about 3 x 0.2–0.3 cm.

Type collected by E. West and W. A. Murrill in soil under gallberry bushes by a lake in Cary Memorial Forest, Alachua Co., Fla., Nov. 19, 1938 (*F 18401*). Having the appearance of *Collybia* but with fleshy stem and very fragile cap. The dark margin is quite distinctive.

Hydrocybe subceracea sp. nov.

Pileo convexo-subexpanso, gregario, 1.5–2 cm. lato, flavo, disco luteo; lamellis subdecurrentibus, sporis 5 x 2.5 μ; stipite concolori, 3–4 x 0.2–0.3 cm.

Pileus convex to subexpanded, with a small broad umbo at times, gregarious, 1.5–2 cm. broad; surface rather viscid, smooth, glabrous, flavous tinged with luteous when young and at the center when mature; margin even, entire to slightly lobed; context very thin, flavous, odorless, mild; lamellae arcuate, short-decurrent, rather broad, inserted, medium distant, entire, pale-yellow, unchanging; spores subellipsoid, obliquely apiculate, smooth, hyaline, about 5 x 2.5 μ; cystidia none; stipe subequal, often flattened, smooth, glabrous, concolorous or paler yellow, 3–4 x 0.2–0.3 cm.

Type collected by E. West and W. A. Murrill under hardwood trees at Newnan's Lake, Fla., Nov. 15, 1938 (*F 18374*). Near *H. ceracea* (Wulfen) P. Karst. but not striatulate and with different gill structure and smaller spores.

Hygrophorus russuliformis sp. nov.

Pileo convexo-depresso, 5–7 cm. lato, viscido, vinoso; lamellis angustatis, confertis, albis; sporis elongatis, 8–12 x 2.5 3.5 μ; stipite subconcolori, 3–4 x 1.5–2 cm.

Pileus convex to depressed, gregarious, 5–7 cm. broad; surface viscid, fibrillose-squamulose, vinosous at the center, lilac with vinose streaks on the margin, which is even, entire to undulate, incurved when young; context thick, firm, white, unchanging, odorless, mild; lamellae adnexed, rounded behind, narrow, inserted, crowded, entire, white, slightly purplish where bruised; spores oblong, obliquely apiculate, smooth, hyaline, granular, 8–12 x 2.5–3.5 μ; cystidia none; stipe enlarged below, striate, solid, white at the apex and within, white streaked with vinosous below, about 3–4 x 1.5–2 cm.

Type collected by Erdman West under an oak at Newnan's Lake, Nov. 16, 1938 (*F 18404*). Suggesting *Melanoleuca Russula* but with totally different spores. The dried specimens have a decidedly agreeable odor, like coconut candy.

Hygrophorus subsordidus sp. nov.

Pileo convexo-subdepresso, 6–8 cm. lato, viscido, albo; lamellis adnexis, distantibus, sporis 6 x 2 μ; stipite albo, 3–4 x 1–2 cm.

Pileus convex to slightly depressed, gregarious or scattered, 6–8 cm. broad; surface viscid but not slimy, smooth, glabrous, white, margin even, undulate or lobed; context thick, watery, white, odorless, mild; lamellae adnexed, narrow, inserted, distant, a few forked midway, entire, white, unchanging; spores cylindric, smooth, hyaline, granular, about 6 x 2 μ, some 7 x 3 μ; cystidia none; stipe tapering downward, smooth, slightly pruinose at the apex, glabrous below, white, unchanging, 3–4 x 1–2 cm.

Type collected by E. West and W. A. Murrill in oak-pine woods at Newnan's Lake, Fla., Nov. 15, 1938 (*F 18403*). Closely resembling *H. sordidus* Peck but with narrower gills and slenderer spores.

NEW COMBINATIONS

For those using Saccardo's nomenclature the following new combinations are made:

Cortinellus formosus = **Tricholoma formosum**
Hydrocybe subceracea = **Hygrophorus subceraceus**
Melanoleuca adusta = **Tricholoma adustum**

HERB. FLA. AGRIC. EXPER. STATION,
GAINESVILLE, FLA.

NEW OR NOTEWORTHY BASIDIOMYCETES

By W. C. Coker

Plates 34–44

Sarcodon piperatus n. sp.

Plates 34 and 44

Gregarious, sometimes fused either in stem or cap; cap up to 7.5 cm. broad, tending to be rather evenly circular but often more or less lobed, especially when fused with others; surface convex to plane with a central depression, delicately pruinose when young and remaining so on the whitish margin of older plants, smoothish or moderately scrobiculate in center and at maturity with radiating fibrous-looking ridges; color light straw drab, usually with zones of darker color toward the margin when mature; young plants not zonate Flesh not duplex, about 6–7 mm. thick near stem and 3 mm. thick at the blunt sterile margin, markedly concentrically zonate, especially toward margin, concolorous, firmly fleshy-fibrous and elastic but easily splitting radially. Taste strongly peppery; odor very faint, suggesting some disinfectant, faintly of fenugreek in No. 10682.

Spines 1.5 mm. long or less, densely crowded, pale drab brown then darker, tips remaining whitish indefinitely, strongly decurrent over entire exposed surface of stem.

Stem irregular and usually more or less crooked, firm, solid, up to 2 cm. thick in center, often somewhat enlarged at the insertion, broad and bluntly rounded below; no obvious rhizomorph; surface where not covered with spines concolorous with cap. Flesh homogeneous, firmer than in cap, concolorous, with distinct horizontal zones of darker color and interior near base often darker with blackish brown grub-channels. All parts turn brown when bruised. In No. 10682 flesh of stem had a vinaceous tint and that of cap was olive in places.

Spores (of No. 10683) brown with vinaceous tint (fawn color, Ridg.), subspherical, distinctly warted, 4.2–5.5 μ. Basidia 4-spored, clavate, 5–6.5 μ thick.

This species is clearly marked among the Sarcodons and fleshy Hydnellums by its pale straw drab zonate cap, almost glabrous; very thick margin; very short, drab brown, strongly decurrent teeth; thick blunt stem base; and strongly peppery taste when fresh. It resem-

bles *H. amarescens* in the peppery taste, but easily differs in color of cap and flesh, very thick margin, blunt stem with usually enlarged base, and usual absence of fenugreek odor.

North Carolina. Chapel Hill. No. 10683 (type, Univ. N. C. Herb.; also painting). In cool deciduous woods, Sept. 22, 1937. No. 10682. Mixed woods, Sept. 20, 1937. Spores distinctly warted, subspherical, 3.7–4.5 x 4–5 μ. No. 10687. In woods, Sept. 23, 1937.
Highlands. No. 12249. In deciduous woods, Aug. 28, 1939. Spores warted, 4–5.4 μ thick.

Sarcodon cristatus (Bres.) n. comb.

In open colonies or at times connate, convex to expanded, usually depressed in center, lobed and irregular, about 3–10 cm. (rarely 19 cm.) broad, when young covered with a creamy white plush, then becoming strongly strigose hairy except near the margin, at times (after heavy rains) this hair collapsing into a spongy pitted layer, color buffy brown to buffy straw or yellowish. Flesh up to 5 mm. thick near stem, firm, elastic, concolorous, zoned, not duplex except for collapsed hairs; odor strongly farinaceous, taste same at first, then soon quite peppery and astringent.

Teeth when young brownish straw color, soon darker and then very dark, almost blackish brown with tips paler, up to 5 mm. long, shorter ones intermingled, close, terete, strongly decurrent.

Stem centric or excentric, about 2.5–4 cm. long, 7–15 mm. thick, somewhat larger below or nearly equal, at times compound; surface concolorous with the appearance of leather, not thickened by spongy tissue, base blunt.

Spores (of No. 10609) brown with vinaceous tint (good print), distinctly warted, subspherical, 3.8–4.2 x 4.2–5 μ.

Banker (Mycologia **5**: 13. 1913) thinks this is the same as *Hydnum acre* Quélet (Bull. Soc. Bot. Fr. **24**: 324–XXXII. 1877), but the latter's figure does not suggest our plant, and Rea's description (Brit. Basid., p. 632) differs from ours in a number of important characters. For photograph and spore drawings of the American plant, see this Journal **41**: pl. 52, and pl. 64, fig. 3, 1926.

North Carolina. Chapel Hill. No. 10672. In cool ravine, deciduous woods, Sept. 19, 1937.
Highlands. No. 9019. On Mt. Satulah, July 31, 1931 (Hesler, coll.). Spores subspherical, warted, 3.8–5 μ thick. No. 9831. On a buried root, Aug. 27, 1934. Spores rosy brown in thin print; subspherical, warted; 4–5 x 4.5–5.8 μ. No. 10609. In rich humus of Kalmia-Rhododendron woods, Aug. 28, 1937.
Also Nos. 9022, 9652, 9658, 9727, 10622, 10920, 12330 (in our herbarium.)

Sarcodon roseolus Banker

Plate 44

In my treatment of *Hydnum amarescens* (Jour. E. M. Sci. Soc. **41**: 274. 1926) I considered *Sarcodon roseolus* as a synonym. Our finding of good fresh specimens of this species in the mountains of this state near the type locality shows that the two species can easily be separated by the mild taste, very thin flesh, much shorter spines, and paler, more rosy color of the latter [Banker, not having fresh plants, does not mention taste or odor].

A description of *S. roseolus* follows:

Cap 2.6–3 cm. wide, plane, the center depressed, the thin margin incurving; surface dry, subshining, light vinaceous rose color, minutely tomentose-squamulose; context homogeneous, fleshy but not brittle, only up to 4 mm. thick, concolorous, deeper vinaceous near stem; taste mild, odor faintly of fenugreek.

Spines very short, up to 0.7 mm. long, not very close, concolorous with pale tips, then darker brown, slightly decurrent, soon fading to dots.

Stem 3–3.5 cm. long, 4–5.5 mm. thick in center, tapering downward, base pinched to a sharp point, concolorous above, darker below, the sharp base deep blackish green, surface of stem glabrous to subpilose, punctate above by aborted teeth.

Spores (of No. 10991) "pale-brown" (Banker), irregularly angled and warted, subglobose in general outline, 3.8–5 x 4 6 μ.

A beautiful and delicate little plant which seems to be very rare. In the dried state it is distinctly lilac brown all over, including the flesh.

North Carolina. No. 10991. Pisgah Forest, Big Bear Pen Creek, Aug. 23, 1938.

Sarcodon brevipes n. sp.

Plates 35 and 44

Cap up to 12 cm. broad, centric or excentric, depressed in center with expanded margin drooping and irregularly lobed and sterile for about 2 mm., surface quite smooth and glabrous, dull, grayish brown in center with somewhat reddish tint, much paler toward margin which is brownish straw, zoned with color. Flesh thin, homogeneous, nearly white, slowly turning light brown when cut, up to 7 mm. thick near stem, firmly fleshy, almost tasteless and odorless; brittle when dry.

Teeth close, light cream when very young, becoming slowly clear brown with pale tips, total effect gray-brown, short, up to 2–3(–4) mm. long, decurrent, in some cases all the way to the ground on the side.

Stem rather short, about 4–5 cm. long and 1–2.2 cm. thick, quite irregular in both shape and thickness, crooked, base bent and pointed; flesh very firm, concolorous with that of the cap except in the bent base where it is *light smoky lilac*, becoming darker after being cut; also light tints of lilac in the cut stem above the base after standing a while. There are several distinct horizontal zonations in the stem flesh.

Spores (of No. 10253) brown, bluntly and irregularly warted, 3.6–4.2 x 4–5.2 μ.

This species, both in the fresh and dried state, approaches nearest *Hydnellum humidum*, from which it differs in the much larger and fewer warts on the spores, smooth cap, in the pointed stem base with its smoky lilac flesh, and in the change to light lilac of the stem flesh throughout after being cut; also the spines are at least half again longer and are decurrent over the whole exposed part of the stem in all four specimens of the collection.

North Carolina. Highlands. No. 10253 (type, U. N. C. Herb.). Under hemlock hedge by Ravenel Lake, Aug. 28, 1936.

Sarcodon gravis n. sp.

Plates 35 and 44

Plant irregularly expanded, tending to be disk-shaped, excentrically attached by a thick root which tapers gradually or quickly into a thread-like rhizomorph. Cap large, heavy, up to 14 cm. broad, surface when quite fresh grayish fawn in center, pure white toward margin, which becomes more or less light yellow on exposure; surface more or less nodulose and pitted but not harshly scrobiculate, delicately velvety, not zonate; in old plants central region becoming very dark brown. Flesh not duplex, up to 3 cm. thick in center, tapering toward margin which suddenly becomes a mere membrane, sometimes inrolled in drying, pallid translucent mottled with more opaque areas when soaked, becoming deep olive green toward center, very distinctly zonate from margin to stem, passing into the firmer deeper bluish black root, which is up to 2 cm. thick at top and tapering into a single ropy rhizomorph about 1.5 mm. thick; taste none, odor faintly aromatic, not unpleasant.

Teeth densely crowded, up to 1 cm. long, not very sharp, pale grayish brown when young, tips white, becoming dark brown then blackish in age, turning brown when rubbed.

No true stem present, the plant being sessile on a subterranean root.

Spores (of No. 10563) a peculiar dun color or buffy drab, irregularly and bluntly warted, 3.6–4.2 x 4–5.2 μ.

North Carolina: Highlands. No. 10563 (type, U. N. C. Herb.). By road around Ravenel Lake, Aug. 19, 1937.

Hydnum albidum Peck. Bull. N. Y. St. Mus. 1, No. 2: 10. 1887.

Plates 36 and 44

In the Mitchell Journal for April 1926 we discussed the confusion in regard to *H. albidum*. Since publishing this note, we have through the kindness of Dr. Homer D. House examined the spores from all the plants of *H. albidum* in the type collection from Sandlake, N. Y., at Albany, and we find that in some way there has been a mixture with larger spores. However those taken from the teeth were small, agreeing with Peck's measurements. Further collections and studies at Highlands, N. C., clearly indicate the distinction between *H. albidum* and *H. repandum.*

A full description of the true *H. albidum* as it occurs in our mountains follows.

Cap 1–7 cm. broad, more or less plane, irregular, often lobed, hygrophanous, not viscid, delicately felted, becoming smooth, nearly chalk white at first, then creamy with orange stains where rubbed. Flesh up to 7 mm. thick near stem, pure white, turning orange when cut, firm but tender; taste quite mild, odor none.

Teeth up to 6 mm. long, white, sharp, close, often decurrent on one side but not all around.

Stem very variable in thickness and length, such as 5 x 1 cm., 5 x 2 cm., 2 cm. x 3 mm., etc., usually excentric, sometimes central or when growing on banks often quite lateral, crooked and irregular, surface glabrous; flesh like that of cap, quite solid, white but like all other parts quickly turning buffy orange when cut or rubbed; base of stem rounded, not rooting.

Spores (of No. 8931) pure white, small, oval, smooth, 3.5–4 x 4–5.2 μ.

The species differs from *H. repandum* in the much smaller spores (spores of the latter about 5.5–6.8 x 7–8.5μ), usually much smaller size of plant, and whiter color. In both species all parts turn orange when rubbed or crushed. Quélet has described a white form of *H. repandum* as var. *album.* To this we have referred a plant from Tennessee (Hesler, No. 10984).

North Carolina. Highlands. No. 8782. In mixed woods above Harbison's Lake, July 20, 1931. No. 8836. By Ravenel Lake, July 23, 1931. Spores 3.4–3.7 x 3.7–4.2 μ. No. 8852. By Ravenel Lake under rhododendrons, July 23, 1931. No. 8931. As above, July 27, 1931. No. 9000. In low mossy woods, July 30, 1931. No. 12002. In rich soil by Ravenel Lake, under hemlock and rhododendron, July 9, 1939. Spores pure white, about 3.7 x 4.5 μ.

Transylvania County. Little Toxaway Falls, No. 7635. Under overhanging rocks, July 3, 1926. Spores 3.6–4.2 x 4–5.4 μ.

Phellodon Hesleri n. sp.

Plates 37 and 44

Cap up to 12 cm. broad, more or less concave, much lobed and often with proliferating caps from any point on the surface, marginal ring of new growth up to 1.5 cm. wide, pure white when quite fresh, soft felted, taking the imprint of a finger, older areas deep brown to buffy brown and subshining. Flesh of lobes zonate, thin, 3-3.5 mm., spongy surface layer very thin except on growing marginal area where it is up to 3 mm. thick, hard layer brown; taste none, odor when fresh strong but not unpleasant, variously judged as that of melilot or slippery elm, when dry odor of fenugreek. When dry the plant is rigid-elastic and not very brittle.

Spines short, up to 2.5 mm., blunt, dense, nearly white, then pale gray-brown.

Stem subcentral or by abortion lateral, brown, stout or rather slender, up to 3 cm. thick above ground, the blunt base more or less enlarged by the spongy layer, which above the substratum is very thin; central core hard and concolorous.

Spores (of No. 12240) pure white, minutely spiny-warted, subspherical, 3.7–4.5 μ thick.

This striking plant is nearest *Phellodon putidus*, from which it differs in the much thinner spongy layer, barely more than a membrane except on the growing margin, the subshining surface except on margin, instead of the dull plushlike surface of *P. putidus*, and larger size and more proliferations, as a rule. Another peculiar difference is that in *putidus* the very thin hard layer of cap and stem is in almost all collections largely channelled by grubs, leaving it almost black. This is not the case in the present species. *Phellodon putidus* is also far less conspicuously zonate except in rare cases, usually not zonate at all, whereas the present species has always a conspicuous zone where the soft surface layer of the margin has collapsed. The odor of *putidus* is more unpleasant.

We take pleasure in naming this species for Dr. L. R. Hesler of the University of Tennessee, who brought in the first specimen in 1932.

North Carolina. Highlands. No. 12240 (type, U. N. C. Herb.). Under dense rhododendron by road near Shortoff Mt., Aug. 27, 1939. No. 3498 (Hesler, coll.). On soil, Mt. Satulah, Sept. 4, 1932. Small, delicate specimen. No. 12382 (Hesler, coll.). On soil, frondose woods, Mt. Satulah, Aug. 31, 1939.

Hydnellum rhizopes n. sp.

Plates 38 and 44

Plants medium to small, usually confluent above or throughout to near the root, more or less amorphous, expanding upward; individual caps up to 5.5 cm. broad, surface nearly plane to depressed in center, not zonate strigose-hairy to spongy-tomentose, irregular and pitted, reddish brown, margin blunt, paler when fresh and quickly turning blackish when bruised. Flesh concolorous, not zonate, duplex, the spongy layer thick and extending deep into the center, hard layer very thin; odor and taste pleasantly acid, no fenugreek.

Teeth short and stout, up to 2.5 mm. long, sharp-pointed, strongly decurrent, color of cap, marginal ones paler.

Exposed stem short, irregular, usually complicated by fusions, individuals up to 1.3 cm. thick, deeply penetrating the humus, surface not spongy but flesh firm throughout except in the rooting part which is covered with a thin paler spongy layer, not in any sense really bulbous, tapering at the end to a small white rope-like strand.

Spores (of No. 8897) pale brownish under the microscope, irregularly angled and warted (relatively few warts in outline view), 4–5.4 x 5–6.2 μ, with a single distinct oil drop.

North Carolina. Highlands. No. 8897 (type, U. N. C. Herb.). In chestnut-hemlock mountain woods, July 24, 1931.

Hydnellum longidentatum n. sp.

Plates 39 and 44

Cap flabelliform from a rather distinct, irregular lateral stem about 4 cm. long, 1 cm. thick, 2 caps from 2 stems fused below, the caps themselves fused laterally but not below and proliferating at the area of fusion (about 4.5 cm.) into a fresh new cap without a stalk; total spread of both caps 13 cm. Individual cap expanded, 6.5–7.5 cm. wide, about 4.5–5 cm. long, margin blunt, surface much roughened and channelled radially, soft-tomentose on the margin of the fresh cap, soon collapsed into a soft felt; color when fresh pinkish white on the margin, then buffy pink to darker buffy brown to nearly black in age, the fresh marginal area staining a distinct watery red when rubbed and when quite fresh exuding droplets of the same color. Flesh homogeneous except for the felt, sordid white, very fibrous and elastic, about 4 mm. thick, obscurely zonate horizontally as rings of growth; taste slight; odor mildly fragrant, to most individuals having a combination of celery and fenugreek.

Spines up to 11 mm. long, gradually shorter toward the sterile margin, descending the stems to the point of fusion, very close and remarkable in the fact that the bases are so compacted as to appear fused in older

parts, points sharp, color blackish gray with tint of purple in the fresher parts, the tips not lighter, the purple replaced by brownish in the older parts.

Spores smoky buff, irregularly angled and warted but not spiny, subspherical in general outline to more elongated, 3.5–4 x 4–4.8 μ.

A remarkable species. The two older caps as well as the younger one were still throwing spores. On the side of one of the older caps are two additional small proliferations, also with young spines. The growing cap grows around twigs as in most Hydnums. This is a *Hydnellum* but the form is different from any we have seen. It is strictly flabelliform with a lateral stem and proliferating habit. It is apparently annual, forming new caps at favorable growth periods during the season.

This species seems most like *H. humidum*, but the smaller spores with fewer and thicker warts, the long teeth without white tips, peculiar color, red juice, and the form of growth easily distinguish it. Our spore measurements of *H. humidum* in this Journal **34**: 191, 1919, are too small. By careful remeasurement they are 4–5 x 4.2–6μ. The drawing on plate 29, vol. **34**, is correct.

North Carolina. Highlands. No. 9589 (type, U. N. C. Herb.). On an exposed root, lying on the ground in deep rich mixed woods (Rhododendron, hemlock, etc.), Sept. 1, 1932.

KEY TO THE SPECIES OF FLESHY STIPITATE HYDNUMS OF THE EASTERN UNITED STATES*

Spores colored (brown, fawn, etc.), warted (*Sarcodon*)
- Cap distinctly scaly at maturity, dark brown
  - Stem not pointed and not blackish green at base; teeth long (up to 1 cm. when mature) .... *S. imbricatus*
  - Stem blackish green at base
    - Teeth shorter (up to 5 mm.); flesh very bitter .... *S. fennicus*
    - Teeth very short (up to 1 mm., rarely 1.5 mm. when dry); plants usually infundibuliform .... *S. Murrillii*
- Cap hairy at maturity, color tan to ochraceous; spores 3.7–4.8 x 4–5.5μ .... *S. cristatus*
- Cap inherently scaly (no free scales except delicate ones in center), light brown to fawn; stem tapering to a fine point which is pure white at base; teeth up to 2 mm. long; flesh mildly bitter; spores 5.5–6.5 x 6.3–7.8μ .... *S. Underwoodii*
- Cap smooth to pubescent or felted, not scaly (but may be channelled in some species)
  - Young teeth violet .... *S. fuligineo-violaceus*

---

* *Sarcodon Blackfordae* from New York and *S. atroviridis* from Alabama are omitted, as I have not seen them.

Young teeth not violet
Base of stem thick and blunt
Spores over 6.5μ thick; taste mild or bitterish, not strongly peppery; plant usually large
Cap pale flesh color...*S. scabripes*
Cap grayish tan to drab..*S. fumosus*
Cap brown...............*S. imbricatus* (old smooth forms = *S. laevigatus*)
Spores less than 6.5μ thick
Taste strongly peppery...*S. piperatus*
Taste not peppery
Teeth short, up to 5 mm., their tips pale...........*Hydnellum humidum**
Teeth long, up to 11 mm., their tips concolorous........*H. longidentatum*
Base of stem tapering to a slender, smoky purple or smoky green root
Flesh of cap and stem vinaceous
Taste strongly acrid and astringent........................*S. amarescens*
Taste mild...*S. roseolus*
Flesh of cap not vinaceous (stem flesh may be tinted lilac)
Flesh thin (up to 7 mm.), not zonate, whitish................. *S. brevipes*
Flesh thick (up to 3 cm.), zonate, greenish toward center........*S. gravis*
Spores white, warted or echinulate; cap not scaly (anomalous)†
Plants large, heavy; teeth 5–8 mm. long...*S. fuligineo-albus* (= *S. reticulatus*)
Plants smaller but firm and solid; teeth 1–2 mm. long......*Phellodon carnosus*
Plants very small, soft and delicate; teeth up to 1.5 mm. long....*P. delicatus*
Spores white, smooth; cap not scaly (*Hydnum* and *Sistotrema confluens*)
Cap white; plant large and heavy; stem very short; spores subspherical, about 6.5–8μ.......................................*Hydnum albo-magnum*
Cap buff to cream, turning orange when rubbed; plants usually of medium size; spores subspherical, about 6.5–8μ..............................*H. repandum*
Cap white to cream, turning orange when rubbed; plants small to medium; spores 3.3–3.7 x 3.8–5.2μ...*H. albidum*
Cap white, buffy when rubbed, very small and delicate; hymenium of irregular plates and teeth; spores very small, about 2 x 4.5μ .. *Sistotrema confluens*
Spores pink, warted (*Hydnodon*)
Cap not scaly, soft and delicate, very thin..........*Hydnodon thelephorum*

Coltricia Memmingeri Murrill

Plates 40 and 44

In the Bulletin of the Torrey Botanical Club **31**: 347, 1904, Murrill described *Coltricia Memmingeri* from clay banks at Blowing Rock, N. C., and it has not been reported since. He described the cap surface as "ornamented with long imbricated scales." When I first found the plant at Highlands, N. C., in August 1938, I could not refer it to *Mem-*

* The distinction between *Sarcodon* and species of *Hydnellum* with flesh not duplex is unsatisfactory.

† See note under *Phellodon carnosus* (this Journal **41**: 277. 1926).

mingeri as the cap had no sign of scales but was plush-like as shown on plate 40 (below). Of the four later collections, three were like the first and one had scales as described by Murrill (pl. 40, above). In all characters except the presence or absence of scales, all these collections were alike. In September of 1939 I compared Murrill's type at the New York Botanical Garden with those from Highlands. We could find only one plant but this was marked "type." The surface was not shaggy but quite smooth like all of our collections except one. Spores of the type were larger than the measurements given by Murrill and agreed with those of our plant.

A description of the species as we find it follows:

Plant massive, irregular, often confluent-complicated. Cap thick, ill defined from the stem, subcircular to irregularly lobed, surface convex or irregularly plane, nearly even or channelled and tuberculate, usually smooth and plush-like to tomentose, less often with conspicuous suberect fibrous scales over most of the central region; color deep russet brown except for the much paler growing margin which is blunt and turns deep brown when rubbed. Flesh concolorous, not zonate, soft-corky above, becoming gradually firmer downward and somewhat darker in the stem, strongly bibulous.

Tubes up to 5(–8) mm. long, mouths very irregular, often radiate-elongated, large, often jagged, whitish when fresh, then brownish drab, small ones on margin *color of cap*.

Stem very variable, more or less amorphous, at times compound, sometimes deeply rooted and with irregular thickenings underground, again spreading out at the base on the surface of the soil into a thin disk with the surface texture of the cap. This disk may have no extension into the soil except the delicate mycelium.

Spores (of No. 11011) brownish, oval to short-elliptic, smooth, 4.4–4.8 x 8–9.3 μ.

North Carolina. Highlands. No. 11011. In rhododendron woods near a spring, Aug. 30, 1938. No. 12082. Same spot as above, Aug. 4, 1939. No. 12092. On bank by road, Ravenel Lake, Aug. 5, 1939. No. 12212. On bank at foot of Sunset Rock, Aug. 22, 1939. Spores smooth, oval, no visible mucro, 4.8–5 x 8–9.3 μ. No. 12252. On gravelly bank by road, Aug. 29, 1939. Spores 4–4.5 x 8–9.3 μ.

Clavaria vernalis Schw.

Plate 41

Since the publication of our book on Clavarias, one of our students, Mr. James Doubles, has found a populous colony of this species about two miles north of Pinehurst, N. C. (No. 11059, Nov. 13, 1938). The

plant was growing on a sandy bank, associated as usual with a unicellular alga and moss protonema, and the delicate mycelial threads about 1.5–3μ could be seen surrounding the algal cells, as before illustrated by us (Clavarias, pl. 92, figs. 3–7).

Peck's illustration, as *C. clavata,* is a drawing and Burt's is a photograph of the dried plant. As no photograph of the fresh plant has been published, we herewith include one.

Clavaria constans n. sp.

Plate 44

Plant narrowly club-shaped, never branched, up to 9 or 10 mm. high, stalk about 2.5-3 mm., watery white, scarcely different from the club except in color, quite glabrous, about 0.5 mm. thick; club enlarging slightly upward to near the top and there about 1 mm. thick, then gradually tapering to a rounded tip; very pale creamy white, subtranslucent; texture toughish and pliable, both stem and club bending on self without breaking. When fresh, base of stem slightly enlarged but when dry no sign of a mat.

Spores white, rod-elliptic, slightly curved, 1.8–2.6 x 7.5–11 μ, most about 8.9 μ long. Basidia 4-spored, about 7.5 x 11 μ. Threads of stem about 3–4.8 μ thick, cells very long; clamp connections not seen.

All the plants in this colony (estimated at about 4000) were quite strict and simple and very nearly the same height; the colony extending for about 18 feet in length and one foot wide, all plants separate and about ½ to ¾ inch (more or less) apart. The smooth bank on which they grew had evidently not been disturbed for some time, probably several years, and had a greasy, pale drab appearance as if some organism might be present, but careful search for an alga in this surface layer revealed nothing but amorphous granular material of a pale yellow color, possibly bacteria, but no growth appeared when efforts were made to culture it on agar.

In drying plants shrink to about 5–6 mm. long and ½ mm. or less thick (much smaller than *vernalis*), dull cartilage color, usually with a little pure white apical point or cap; stem paler.

The species is obviously nearest *C. vernalis* and *C. mucida* which it strikingly resembles in its occurrence in populous colonies and in small size. It differs from both of these in growth on soil unattached to algae, also from *vernalis* in narrower club and much paler color both when fresh and dry, and from *mucida* in more uniform size, strictly simple habit, apex not becoming red or black (white on drying) and in the longer spores.

Highlands, N. C. No. 10451 (type, U. N. C. Herb.). On a clay bank by Kalalanta Trail, July 26, 1937. Nos. 10788 and 11016. Same spot as above, July 12 and Aug. 30, 1938.

Clavaria Pogonati n. sp.

Plates 41 and 44

Plants single in small colonies on earth covered with protonema of *Pogonatum brevicaule.* Sometimes quite simple, but much more commonly branched once, twice, or even three times, sometimes at or near the ground; slender, filiform and subterete throughout except somewhat flattened at the forks, no distinction between base and branches; color pallid white; very pliable and tough; about 1–2.3 cm. high and 3.5 mm. thick, tips acute; flesh solid, of very slender interwoven threads with clamp connections.

Spores (of No. 10907) smooth, subelliptic, often with the proximal end slightly curved, 4.2–5.4 x 11–13 μ. Basidia 4-spored, about 9.5 μ thick.

This little plant adds a fourth species to the well marked *mucida* group, as shown by its association with a living green plant, its separate units in considerable colonies, its tough texture, and its smooth elliptic spores. It differs from *C. mucida* and from *C. constans* in the much taller and more branched habit, large spores and association with the protonema of *Pogonatum.*

North Carolina. Macon County. No. 10907 (type, U. N. C. Herb.). In rich deciduous woods, Coweeta Experimental Forest, elevation 3200–3400 feet, Aug. 12, 1938.

Clavaria rufipes Atk. Ann Myc. **6**: 57. 1908.

Plate 42

This species has been known heretofore only from Ithaca, N. Y., and Blowing Rock, N. C. We published drawings of the spores and basidia in our *Clavaria* book, but the fresh plant body has never before been illustrated. Burt shows a single dried plant (Ann. Mo Bot. Gard. **9**: pl. 6, fig. 43. 1922).

Our Chapel Hill collection adds somewhat to the recorded stature of the plant, and we give below our notes on it.

Plants about 10–15 mm. high, simple clubs about 1.5 mm. thick or branched near the top into two or several antler-like processes with rounded sinuses, flattened upward, tapering to a terete stalk which is

not obviously delimited from the hymenium. Color dull white or straw. Texture fairly tender and easily broken but not brittle.

Spores white, smooth, pip-shaped to oval, 3–3.7 x 4–5 μ. Basidia about 5 μ thick and 23–30 μ long.

The dried plants shrink to mere threads and are very dark. They agree very well with *C. rufipes* except that the base is not rufous.

North Carolina. Chapel Hill. No. 9821. On bare damp soil under a rose bush, Sept. 26, 1934.

Clavaria biformis Atk. Ann. Mycol. **6**: 56. 1908.

Plate 44

Plants often in extensive colonies, varying from quite simple to several times dichotomously forked, rarely irregularly branched, forks open, growth distinctly crooked, very few of the plants being strict; no clearly distinct stem; entire fruiting part compressed except at times in the entirely simple plants; color light buff except the paler tips; texture very elastic, not breaking when bent on self; odor none. When dry the entire plant brown, tips usually paler.

Spores (of No. 10534) pure white, smooth, 2.5–3.4 x 3.7–5 μ. Basidia clavate, 4-spored, 4.8–6.5 μ thick and about 2.6 μ long.

The compressed habit of the entire fruiting part is a peculiar character. The plant has previously been found by Atkinson at Blowing Rock, N. C., and at Ithaca, N. Y.

North Carolina. Highlands. No. 9501. On very rotten deciduous wood and humus, Aug. 13, 1932. No. 10534. In leaf mold by road, Aug. 16, 1937. No. 10567. In humus, mixed woods by trail, Aug. 22, 1937.

Clavaria subcaespitosa Peck. Bull. N. Y. St. Mus. **167**: 39. 1913.

Plates 43 and 44

Plants usually clustered with several slender distinct or partially fused stems arising from the ground; clusters up to 7.5 cm. high and 4.5 cm. broad, branching prevailingly dichotomous with open angles, the lower spreading, upper strict and very numerous, mostly flattened in plane of branching, ultimate branches usually long, tips sharp, color pure translucent white when young, the lower ones soon becoming tan with tints of flesh, stems remaining white and somewhat pulverulent in places. Texture distinctly brittle and delicate, so much so that the plants can hardly be handled without breaking, in this respect differing from all other species we know with somewhat similar habit and appearance. The entire plant when young and upper part when older with

distinctly translucent appearance. Taste mildly woody acrid; odor slightly woody. Bruises or old wounds somewhat fleshy or lavender brown.

Spores pure white, subspherical to more elongated, 3.6–4 x 4–5.6 μ, finely to distinctly warted, more so around the larger end, just as in our illustration of the spores of the type in our Clavaria book. Basidia 4-spored, about 7.5 μ thick, sterigmata short; hymenium homogeneous without cystidia, about 70 μ thick.

The species is distinguished by the tufted habit, extremely fragile texture, translucent white color becoming buffy in places, and by the spores. Our Chapel Hill collection was from a beautiful colony of many tufts arranged more or less in rows in rich humus in a deep cool ravine. It is interesting to note that it grew not far from a flourishing colony of *Medeola virginiana,* a rare plant so far from the mountains. *Clavaria caespitosa* has been known heretofore only from Massachusetts and Vermont. It has not been illustrated in the fresh state. For the dried plants, see Burt (Ann. Mo. Bot. Gard. **9**: pl. 7, fig. 54).

North Carolina. Chapel Hill. No. 10653. In rich hillside woods, mostly deciduous, west of University Lake, Sept. 10, 1937.

Jackson County. No. 10931. In damp deciduous woods, Whiteside Cove, Aug. 17, 1938. Spores subspherical, minutely warted, about 4 x 4.5 μ.

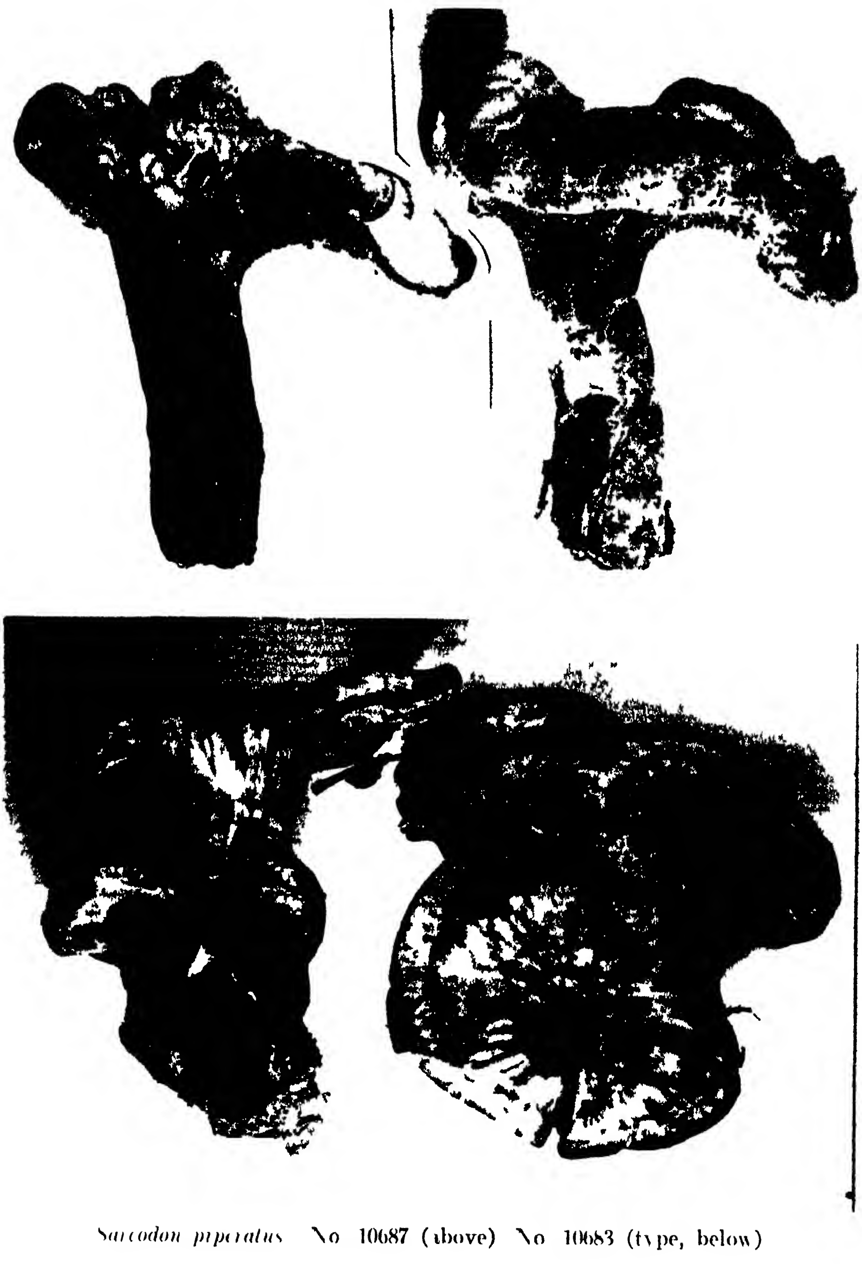
DEPARTMENT OF BOTANY,
UNIVERSITY OF NORTH CAROLINA,
CHAPEL HILL, N. C.

EXPLANATION OF PLATE 44

Figure 11 by Leland Shanor; all others by Alma Holland

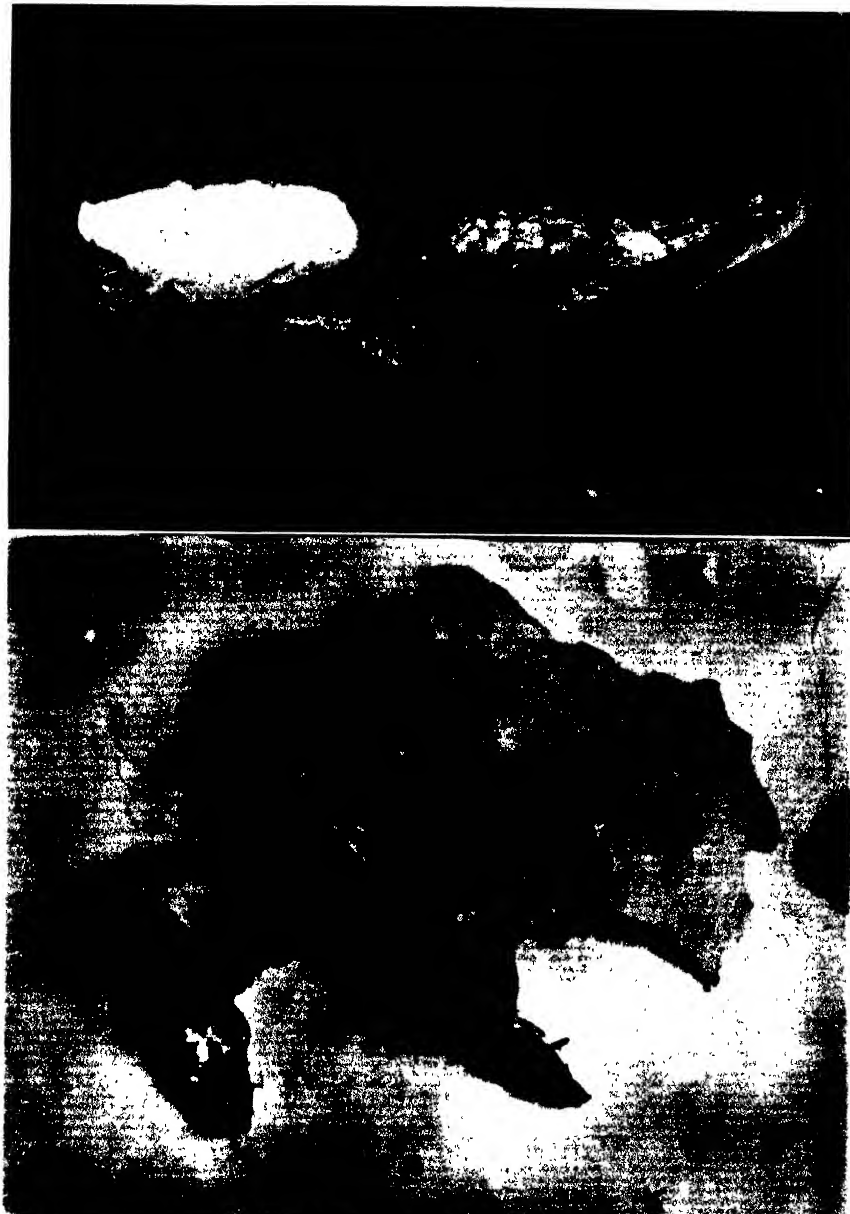
Fig. 1. Spores of *Sarcodon piperatus*. No. 10683. × 1620.
Fig. 2. *S. roseolus*. No. 10991. × 1620.
Fig. 3. *S. brevipes*. No. 10253. × 1620.
Fig. 4. *S. gravis*. No. 10563. × 1620.
Fig. 5. *Hydnum albidum*. No. 8931. × 1620.
Fig. 6. *Phellodon Hesleri*. No. 12240. × 1620.
Fig. 7. *Hydnellum rhizopes*. No. 8897. × 1620.
Fig. 8. *Hydnellum longidentatum*. No. 9589. × 1620.
Fig. 9. *Coltricia Memmingeri*. No. 12212. × 1620.
Figs. 10 and 11. Basidia of *C. Memmingeri*, showing variations. × 810. Fig. 10, No. 11011 (smooth cap); Fig. 11, No. 12212 (scaly cap).
Fig. 12. *Clavaria constans*. No. 10451. × 1620.
Fig. 13. Habit sketch of *C. constans*. No. 10451. × 3.4
Fig. 14. *C. Pogonati*. No. 10907. × 1620.
Fig. 15. *C. biformis*. No. 10534. × 1620.
Fig. 16. Habit sketch of *C. biformis*. No. 10534. Reduced one-fourth.
Fig. 17 *C. subcaespitosa*. No. 10653. × 1620.

PLATE 34



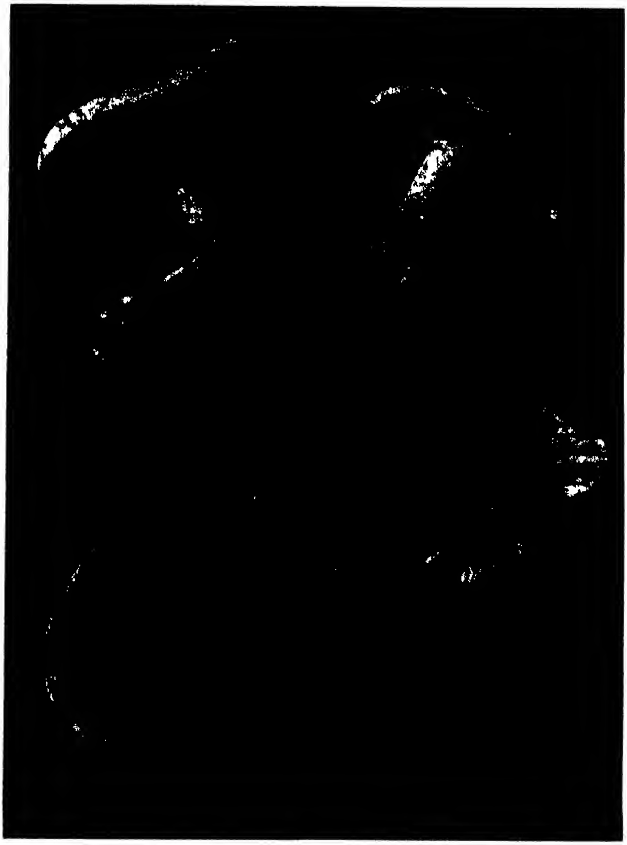
Sarcodon piperatus No 10687 (above) No 10683 (type, below)

PLATE 35



(Above) *Sarcodon gravis.* No. 10563. × ½
(Below) *Sarcodon brevipes.* No. 10253. × ½

PLATE 36



Hydnum albidum No 12002

PLATE 37

Phellodon Hesleri No 12240 (type)

PLATE 38

Hydnellum rhizopes. No. 8897

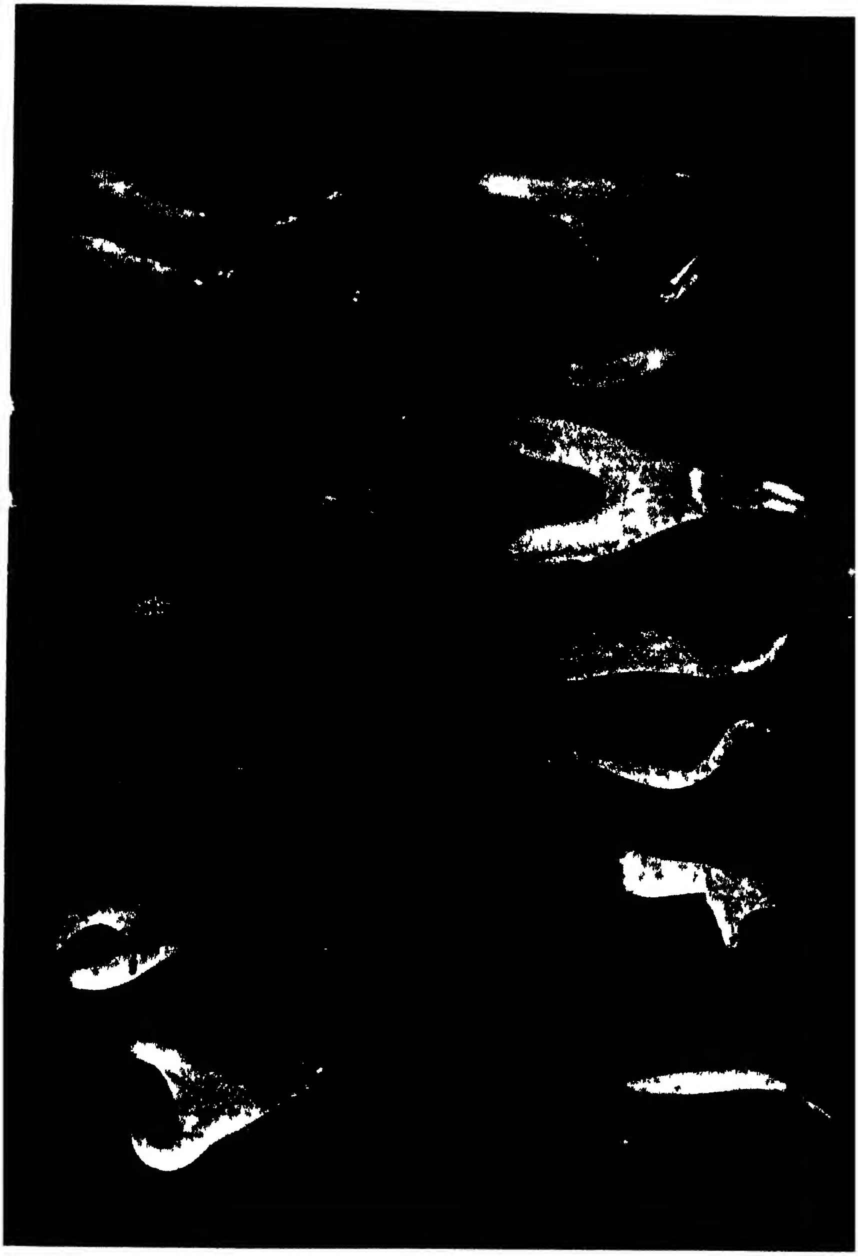
PLATE 39

Hydnellum longidentatum No 9589

PLATE 41

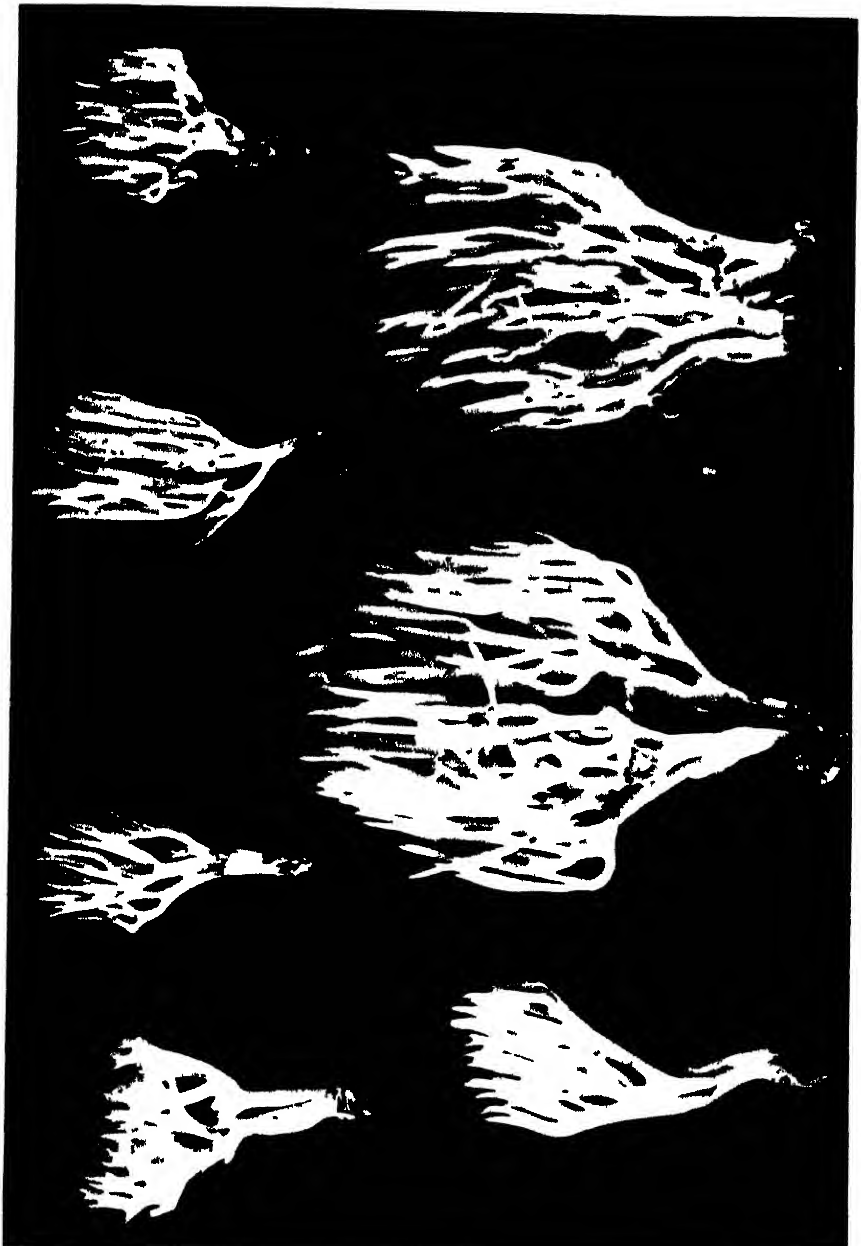
Clavaria Pogonati No 10907 (type, left), No 10908 (right). Nat. size
Clavaria vernalis. No 11059 × 7

PLATE 42



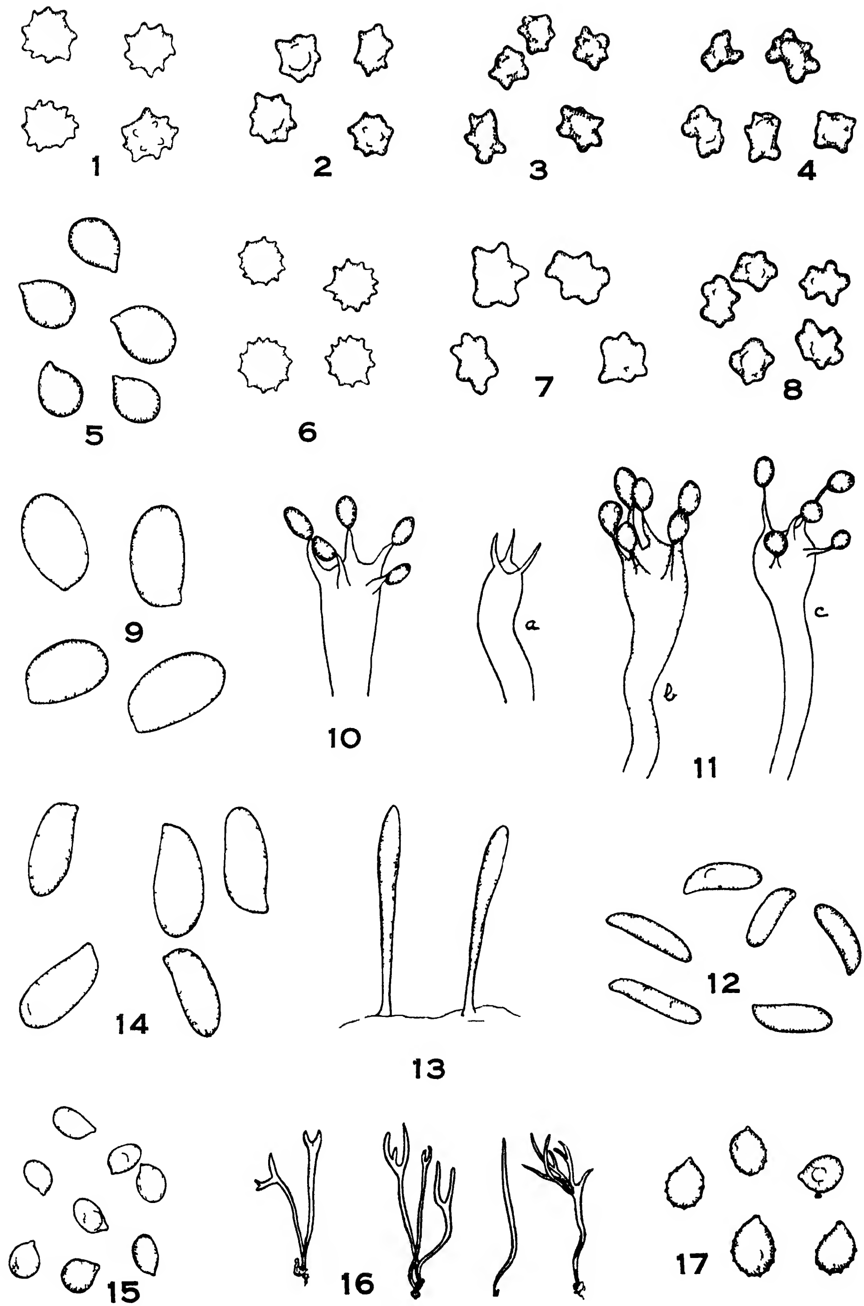
Clavaria rufipes No 9821 × 2

PLATE 43



Clavaria subcaespitosa No 10653

PLATE 44



A NEW GENUS OF THE RHIZIDIACEAE

By Hiden T. Cox

Plates 45 and 46 and One Text Figure

In some collections of water and debris made during the fall of 1938 from the swamps around Florence, S. C., there appeared an interesting chytrid remarkable for its long sporangiophores terminated by a globose or ellipsoid zoosporangium. Moreover, the spores were unusual in that each possessed not a single large refractive body as is the usual case in the chytrids, but contained several globules arranged in a lunate ring toward its posterior.

Certain similarities between this fungus and forms of the Monoblepharidales and the Blastocladiales were immediately apparent, suggesting the possibility that here might be a link connecting these orders with the lower Chytridiales.

The peculiar sporangiophores and spores seemed to warrant the erection of a new genus, tentatively placed in the Rhizidiaceae. To emphasize the club-like appearance of the sporangiophores, the name *Clavochytridium* is proposed. Since entrance into the dead host tissue is effected through a stoma, the species is being named *Cl. stomophilum*.

DESCRIPTION OF GENUS AND SPECIES

CLAVOCHYTRIDIUM Couch and Cox gen. nov.

Thallo monocentrico, eucarpico, rhizomycelio intramatricali atque zoosporangio extramatricali praedito. Zoosporangiis sessilibus vel stipitatis, una vel pluribus papillis dimissionis praeditis. Zoosporis a tergo uniciliatis, duobus usque ad aliquot corpusculis refractis praeditis, plane formatis emergentibus atque mox natantibus. Systema rhizoideorum bene aucta, interdum septate vel constricta; ad maturitatem perducta, pariete transverso ex zoosporangio divisa. Parietibus non caeruleis, *chlor-iodide of zinc* infusis.

Thallus monocentric, eucarpic, with an intramatrical rhizomycelium and an extramatrical zoosporangium. Zoosporangia sessile or stalked, with one or more exit papillae. Zoospores posteriorly uniciliate, with two to several small refractive bodies, emerging fully formed and swimming after a few seconds. Rhizoidal system well developed, sometimes

septate or constricted, delimited from the zoosporangium by a cross wall at maturity. Walls not turning blue with chlor-iodide of zinc.

Clavochytridium stomophilum Couch and Cox sp. nov.

Thallo monocentrico; omne thallo, ad maturitatem perducto, ex crasso rhizomycelio intramatricali atque zoosporangio extramatricali constante. Rhizoideis profusius ramosis, interdum constrictis vel septatis; ramis extremis ultimis partibus obtusis praeditis. Zoosporangiis globosis vel ovatis vel ellipsoideis vel cylindricis, sessilibus vel stipitatis; stipite in longitudine diverso (usque ad 310μ). Sporis singillatim ex una vel pluribis papillis exeuntibus, atque mox natantibus. Sporis a tergo uniciliatis (cilio in longitudine 35 usque ad 50μ), 3.5μ usque ad 6.6μ, globosis vel ellipticis, duobus usque ad duodecim corpusculis excentricis refractis praeditis. Vacuo sporangio hyalino, in superficie hospitis mortui permanente. Sporis perdurantibus non notis.

Thallus monocentric; each thallus at maturity consisting of a coarse intramatrical rhizomycelium and an extramatrical zoosporangium. Rhizoids more or less profusely branched, sometimes constricted or septate, the ultimate branches with blunt tips. Zoosporangia globose, ovoid, ellipsoid or cylindrical, sessile or stalked, the length of the stalk variable (up to 320μ). Spores escaping singly from one or more exit papillae, remaining motionless or becoming amoeboid for a few seconds at the mouth of the sporangium and then swimming away. Spores posteriorly uniciliate (length of cilium 35 to 50μ), 3.5 $\times$ 6.5μ, ovoid or elliptical, with two to twelve small excentric refractive bodies. Empty sporangium hyaline, persisting on the surface of the dead host. Resting spores unknown.

Saprophytic on boiled corn and grass leaves put in water collections from swamp by Kirby's Tavern, 3 miles from Florence, S. C.

This fungus is characterized by the peculiar structure of the spore which lacks the single spherical globule of the typical chytrid, having several small globules instead, the stalked sporangiophores, the lack of cellulose, and penetration through the stomata.

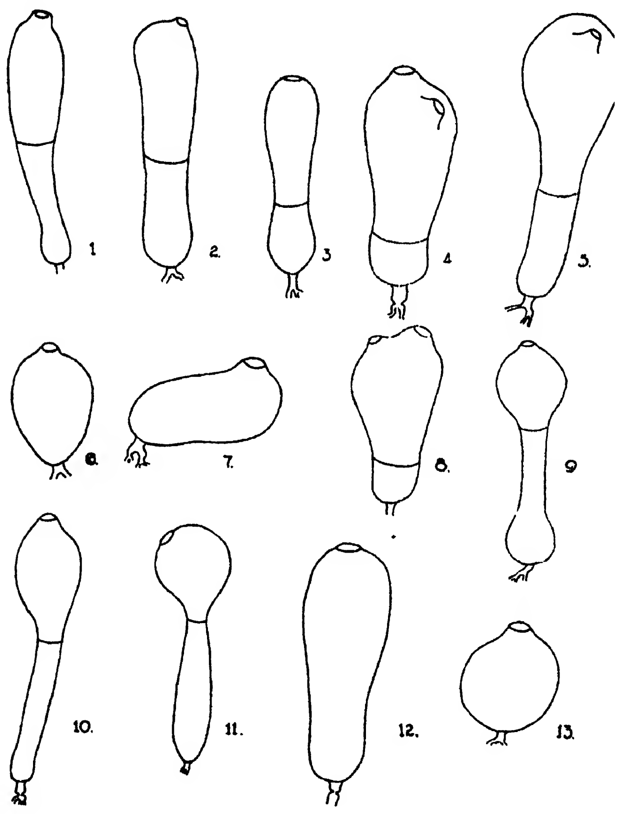
ISOLATIÓN AND CULTURE

The methods used in the isolation and culture of this fungus are essentially those described by Couch (1939).

The fungus was got into pure fungal culture by using the following method: A single sporangium, along with a minute piece of corn leaf, was cut away and carried by means of a sterile needle to a drop of sterile charcoal water in a sterile Petri dish. In the drop of water had previously been placed a small piece of boiled corn leaf. When the spores had been discharged and had begun to germinate on the leaf, the

entire leaf was transferred to another sterile dish with enough water to insure against the desiccation of the fungus.

A more precise technic is the isolation of a single spore on agar. After a single sporangium has been cut away it is carried by means of a sterile needle, or a sterilized capillary pipette, through several successive drops



TEXT FIG. 1. VARIATIONS IN SPORANGIAL SHAPE AND SIZE IN CLAVOCHYTRIDIUM STOMOPHILUM

of water in order to rid it of as many bacteria as possible. During this process the wide field binocular (40 to 100x) was used. After the sporangium has been cleaned it is allowed to remain in the drop of water until the spores have been discharged. Then, using the capillary pipette, a drop of water containing spores is drawn up and blown onto

the surface of a plate of plain agar. To insure wide distribution of the spores the water is made to run the width of the agar plate several times by tilting it back and forth. Ten or twelve hours later the sporangia will have reached such a size that they may be located easily with a binocular, and somewhere on the plate there should be several sporangia free from bacteria. One of these may be cut away with a small cube of agar by using a dissecting needle filed into a spade-shaped tool. This sporangium descended from a single spore is placed in a drop of water containing a leaf of corn and allowed to discharge its spores. Upon spore germination the dish is flooded with water. This culture has the advantage of being both unifungal and bacteria-free.

Another method used successfully is the dilution method by which a single spore may be isolated. As above, a single sporangium is cleaned and placed in a drop of water until the spores have been discharged. While the spores are swimming about, a small amount of the water is drawn up in the capillary pipette. Additional sterile water is added until the volume of water in the pipette is about ten times the original amount. With the aid of the binocular the spores may be made out swimming in the pipette. The pipette may be broken in such a way that a single spore is captured in the broken tube. This may be placed in the usual drop of water with a corn leaf until sufficient time has elapsed to enable the spore to settle down and germinate on the leaf, after which the dish is flooded with water. This culture is unifungal but not necessarily bacteria-free.

The routine method of culturing this chytrid is on boiled, young leaves, in sterile charcoal water (Couch, 1939) in Petri dishes. Corn leaf cultures produced the most luxuriant growth of the Chytrid. Grass and wheat leaves may be used but on such leaves the fungus never grows as vigorously as on corn leaves. Leaves decolorized by boiling in 95% alcohol were used with poor success, the growth being but slight and lasting for only a short time.

The fungus prefers a slightly acid environment and reached its healthiest development in water of pH 5.0 to pH 6.6.

This chytrid has been cultured on various concentrations of plain agar ranging from 0.5% to 4.0%. A concentration of 0.5% to 1.0% plain agar gave best results. The fungus was able to grow and produce spores on the 1.0% agar but due to the solidity of this agar the spores were incapable of protracted activity, which resulted in their settling down only a short distance from the parent sporangium. The sporangia resulting from the germination of these spores were so closely packed

as to cause considerable distortion, and only a few were capable of spore discharge. These usually died from a lack of sufficient room to develop normally. The 0.5% agar gave much better results. This agar has the advantage of being much less firm, permitting a much wider range of motility. Many generations may be completed before crowded conditions bring about the death of the fungus, and transfers may be made from one agar plate to another so that the fungus may be kept in culture indefinitely. Couch (1939) reports the culture of four species of monocentric chytrids, *Rhizidiomyces apophysatus*, *Rhizophidium carpophilum*, *R. multiporum* and *R.* n. sp. on agar in pure culture through several generations. However, he used 2% agar on which the spores were unable to swim, hence the spores when discharged settled down in a mass, the culture dying out after several generations due to overcrowding. For this reason it seems that the less solid agar has a decided advantage.

DEVELOPMENT OF THE FUNGUS

After a period of motility the zoospore comes to rest on a stoma of a leaf of corn, assumes a globose to slightly pyriform shape and loses its cilium, after which it secretes a thin wall about itself. A penetration tube is formed which enters the leaf through the stoma and immediately begins to develop into the rhizomycelial system (Pl. 45, figs. 3, 4, 5). Very soon after entrance the penetration tube may divide into two to several branches.

The rhizomycelial system is well developed (Pl. 45, fig. 1) consisting of a main trunk with numerous smaller branches or of two or several large branches from which the smaller ones arise. The rhizoids branch and rebranch, tapering to very fine, barely visible threads. At maturity the main trunk and the larger branches may sometimes be constricted or septate. There is no evidence of turbinate enlargements on the rhizomycelium, though sometimes the penetration tube may enlarge to form a globose body from which the rhizoids arise.

Simultaneously with the development of the rhizomycelium, the upper part of the spore has been undergoing a distinct change. For the first four or five hours of its development there is little change other than a considerable enlargement of the spore (Pl. 45, figs. 3, 4, 5, 6). The spore now begins to elongate, at the same time becoming more vacuolated and hyaline in appearance (Pl. 45, figs. 7, 8). There is also a noticeable increase in the number of refractive globules. Some of these globules

may fuse to form larger ones, but these oily looking bodies never attain the size of those found in *Rhizophidium* and related genera.

As the sporangiophore elongates the distal half usually enlarges so that a distinct club-shaped structure is formed. Meanwhile the protoplasm accumulates in the swollen part, the basal half becoming vacuolate (Pl. 45, figs. 9, 10). In old cultures in which the sporangia have remained dormant for some time there is discernible not even the slightest vestige of cytoplasm in the zoosporangial stalk, all of it having disintegrated.

A cross wall is now formed cutting off a terminal zoosporangium supported by a stalk (Pl. 45, fig. 11). The sporangium may remain in this condition for several weeks in old cultures, but in fresh clean water the length of this stage is limited, the spores being formed immediately.

Actual cleavage stages were not followed with exactness, but the spores are delimited completely in the zoosporangium and begin a slight rocking motion (Pl. 45, fig. 12). During, or slightly before spore delimitation, one to three or sometimes more, short, apical or lateral emergence papillae are formed.

The immediate indication that the spores are about to be discharged is a rocking and jerking motion in the sporangium. This movement increases until the tips of the papillae disappear and the spores are allowed to make their escape. If there are two or more papillae on the sporangium, the tips do not necessarily dissolve simultaneously, but all will dissolve within a short time.

The spores emerge with their cilia directed backward (Pl. 45, fig. 13). They are detained at the sporangial mouth for a few seconds until each has effected the release of its cilium, which has become entangled in the remaining spore mass. While extricating its cilium the spore may become amoeboid. After the congestion in the sporangium has been relieved the spores may escape more freely, and as the sporangium empties the remaining spores dash about until they find an exit. The time consumed in the evacuation of a zoosporangium is between fifteen and forty minutes.

The normal spore (Pl. 45, figs. 2, 14) is ovoid or ellipsoid in shape, 3.5 x 6.5 μ in size, with a very long (35 to 50 μ) posteriorly attached cilium. The nucleus is placed slightly to the stern end of the spore and is partially surrounded by a lunate ring of from two to twelve small refractive globules. Toward the center of the spore is a very large saddle shaped "food body" which may or may not be placed at right angles to the long axis of the spore.

The spore is able to swim for several hours. If unobstructed it moves along smoothly, rotating slowly about its long axis. If swimming in water littered with debris it appears to follow a jerky course since it quickly changes its course when confronted by an obstacle. While in the sporangium it is capable of amoeboid motion and upon settling down to germinate it may become amoeboid and creep about over the substratum for some time. This is perhaps the means by which it arrives at a stoma prior to forcing in the penetration tube.

Germination on freshly cooked leaves is entirely dependent upon the spore's reaching a stoma. However, in old cultures in which the leaves have become quite soft, the spore is capable of effecting an entrance anywhere on the leaf. Frequently in old cultures the corn leaves are almost covered by zoosporangia or sporangial cases not necessarily confined to the stomatal areas.

DISCUSSION

Certain affinities of *Clavochytridium* with other forms of both the lower Chytridiales and the Monoblepharidales and Blastocladiales are to be noted. In the general organization of the thallus and the method of its development it is chytridiaceous. However, the spore structure and the method of spore discharge seem to indicate clearly its close relation to the Monoblepharidales and the Blastocladiales.

Macrochytrium botrydioides von Minden (1911) may be distinguished from the plant under discussion by its typical chytrid zoospore, the delicate and non-constricted rhizoids of its rhizomycelial system, and the nature of its spore discharge.

Blastocladiella simplex Matthews (1937), another similar form, differs from *Clavochytridium* in that the former always displays the stalked habit of growth, possesses a less highly developed rhizomycelium, forms resting spores and discharges its spores in a vesicle. *Blastocladiella* (*Rhopalomyces*) *variabilis* Harder & Sorgel (1938) shows even more differentiation in that a definite and well ordered alternation of generations is a part of its life cycle.

Of the many species of the Rhizidiaceae described by Karling, Sparrow, Berdan and others there apparently is no form closely related to the plant under discussion.

In attempting to ascertain the position of *Clavochytridium stomophilum* we are immediately aware that it is considerably more advanced than the lower Rhizidiaceae, although it is true that *Rhizophidium globosum* shows a quite marked relationship to it. In the general habit of growth

Rh. globosum is so similar to the sessile forms of *Clavochytridium* that only keen observation enables one to distinguish them. Spore discharge accomplished by the solution of a membrane and followed by the free discharge of the spores is almost identical in the two species. The principal differences, then, lie in the structure of the zoospore and of the rhizomycelial system.

The structure of the zoospore, the method of spore discharge and the lack of rhizomycelial constrictions stamp *Macrochytrium* as being more primitive than *Clavochytridium.*

Clavochytridium is undoubtedly less advanced than *Blastocladiella* since there is in the latter genus an alternation of generations. However *Clavochytridium* is in some respects more suggestive of the higher Blastocladiales and the Monoblepharidales in the structure of the zoospore and the absence of cellulose walls.

The writer is sincerely grateful to Dr. J. N. Couch for his encouragement and guidance during the study of this problem, to Miss Laurie Stewart for assistance in the preparation of the plates, and to Mr. John Oliver for the writing of the Latin descriptions.

SUMMARY

A new genus, *Clavochytridium*, based on the species *Cl. stomophilum* is proposed for a monocentric chytrid, characterized by stalked or sometimes extramatrical sessile zoosporangia and uniciliate zoospores with two to many refractive bodies. No sexual reproduction or resting bodies were observed. Because of its monocentric thallus and epibiotic sporangia it is placed in the Rhizidiaceae, although it apparently is an intermediate form connecting the Chytridiales with the Blastocladiales and the Monoblepharidales. Affinities of this plant to *Rhizophidium, Macrochytrium* and *Blastocladiella* are discussed.

The fungus is apparently saprophytic on leaves in water but has been cultured in pure fungal culture on corn and other grass leaves and in single spore culture on agar. The fungus grew best on plain agar of a concentration of 0.5%, the sporangia maturing and discharging spores which were dispersed on the surface of the agar to repeat the development.

LITERATURE CITED

COTNER, F. B. 1930 Cytological study of the zoospores of *Blastocladia.* Bot. Gaz. **89**: 295–309.

COUCH, J. N. 1939 Technic for collection, isolation and culture of Chytrids. Jour. Eli. Mitch. Sci. Soc. **55**: 208–214.

HARDER, RICHARD, AND GEORG SÖRGEL. 1938 Über einen neuen planoisogamen Phycomyceten mit Generationswechsel und seine phylogenetische Bedeutung. Nachr. von der Gesellsch. der Wiss. zu Göttingen, Math.-phys. Kl., Fachgruppe VI(Biol.), N. F. **3**, Nr. **5**: 119–127.

MATTHEWS, VELMA D. 1937 A new genus of the Blastocladiaceae. Jour. Eli. Mitch. Sci. Soc. **53**: 191–195.

MINDEN, M. VON. 1911 Chytridiineae. Krypt. Fl. Mark Brandenburg **5**: 209–422.

EXPLANATION OF PLATES

PLATE 45

Drawings made with the aid of Spencer camera lucida. All drawings ×535 with the exception of numbers 1, 2, 14, 15

1. Habit of the fungus, showing typical sporangia grouped about stomata of the leaf of corn. Note that one plant has effected entrance through an ordinary epidermal cell. ×200
2. Living spores immediately after spore discharge. ×660

3–6. Early stages in spore germination.

7–12. Later stages showing the formation of a zoosporangium and the delimitation of zoospores.

13. Discharge of spores from the same zoosporangium.
14. Stained zoospore of *Clavochytridium stomophilum*. ×1050. Cotner's (1930) technic.
15. Stained zoospore of *Blastocladiella simplex*. ×1000. Note small globules much as in *Cl. stomophilum*. Preparation and drawing by J. N. Couch from material furnished by Dr. Velma Matthews. Modified Loeffler's cilia stain.
16. Sessile zoosporangium with zoospores congregated at the mouth of the emergence papilla. Note the amoeboid spores.
17. Semi-diagramatic figure of a long stalked sporangium immediately before the delimitation of spores.

PLATE 46

1. Stalked zoosporangia growing on corn leaves. Note the position of the sporangia in reference to the location of the stomata.
2. Sessile zoosporangia growing on corn leaves.
3. Sessile zoosporangium, to the left, discharging zoospores. Right, a large ovoid zoosporangium.
4. Cylindrical zoosporangium terminating a long sporangiophore. To the left an empty sporangium of the same type with two emergence papillae. Center is short-stalked sporangium. Note small, sessile sporangium at the base of the sporangiophores.
5. Young thalli growing on agar, with extent of rhizomycelial growth shown.
6. Typical zoosporangium borne on a short sporangiophore.

PLATE 45

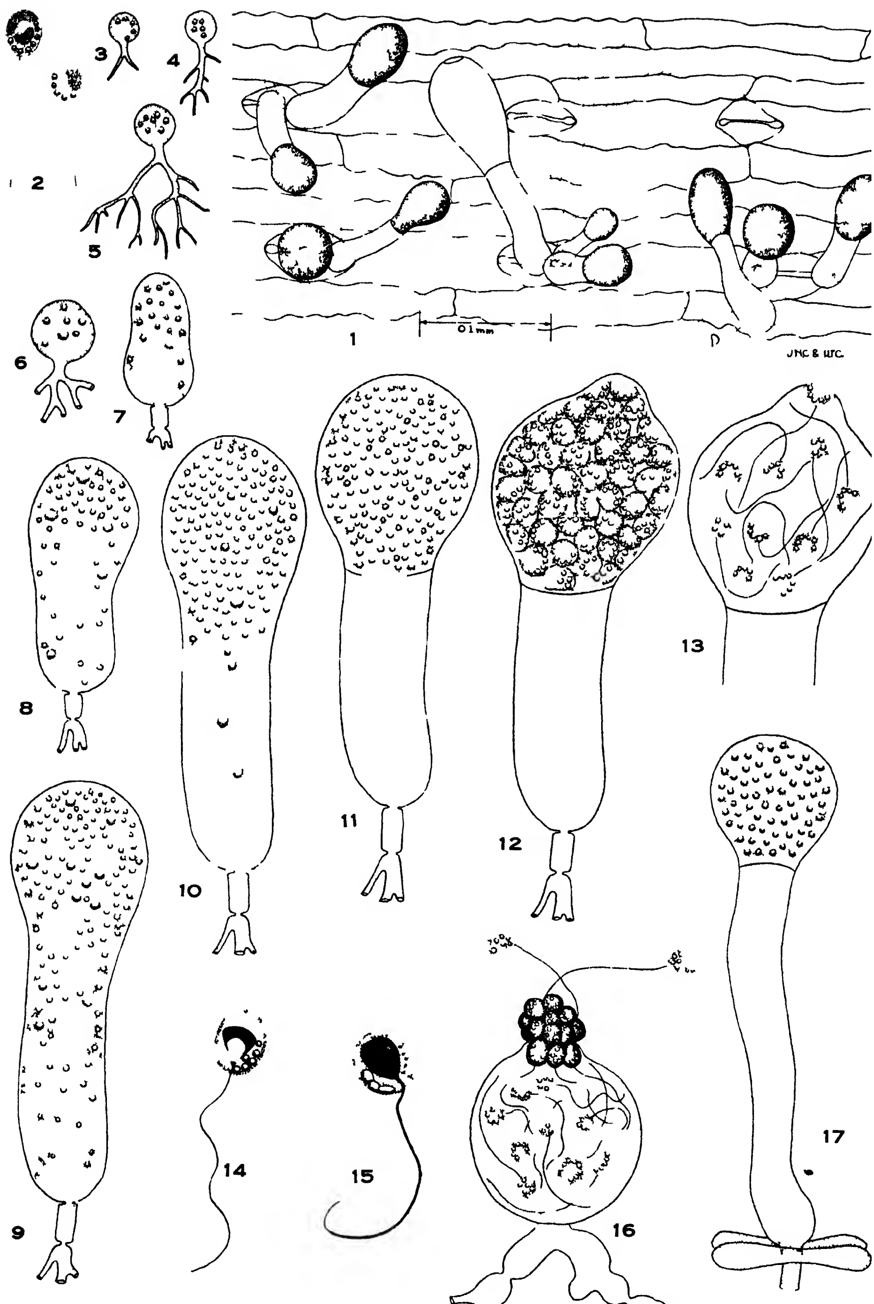
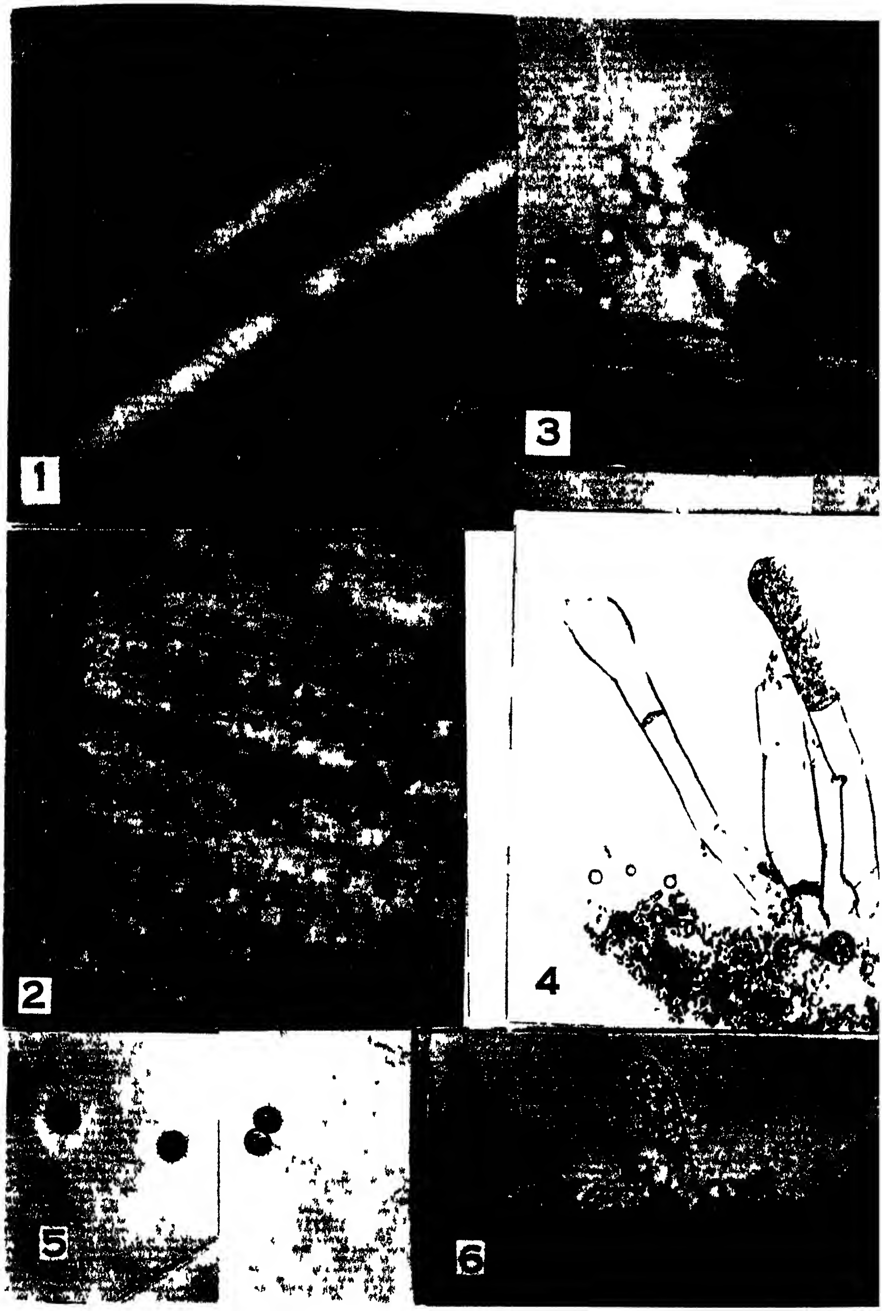


PLATE 46



A NEW GENUS OF THE PLASMODIOPHORACEAE

By J. N. Couch, Jean Leitner, and Alma Whiffen

Plates 47 and 48

From a collection of soil and water made by Miss Frances Foust in the region of Chapel Hill, North Carolina, there was obtained a culture of *Achlya glomerata* Coker, in which the tips of the hyphae were swollen into large, spherical galls, caused by an endophytic parasite. In its early stages of development this parasite appeared within the hyphae as a naked protoplast accompanied by hypertrophy of the host. At maturity the protoplast fragmented into either a sorus of thin-walled zoosporangia or into groups of thick-walled resting spores, the sorus of zoosporangia resembling that of *Woronina polycystis* Cornu. The zoospores were observed to have a long and a short flagellum as has been figured by Ledingham for *Plasmodiophora brassicae* (1934) and *Spongospora subterranea* (1935), and thus it was suspected that the present fungus might belong in the Plasmodiophoraceae as interpreted by Ledingham. Cytological preparations were made during the summer of 1938 using Gram's gentian violet technique as modified by Couch (1932). The nuclei of the vegetative plasmodium were found to be dividing by a peculiar type of nuclear division known as protomitosis. The discovery of the "cruciform division," characteristic of protomitosis, along with the zoospore structure indicated a close relationship to the Plasmodiophoraceae.

The basis of generic distinction in the Plasmodiophoraceae as now commonly agreed upon is the arrangement of the resting spores. The reliability of this distinction, however, has been questioned by Palm and Burk (1933), because of the great variability of the arrangement of the resting spores in the sori of a single species. Certainly before a satisfactory system of classification can be worked out more attention must be paid to the ciliation of the zoospores and the method of nuclear division. Nevertheless, until a better means of generic distinction than the arrangement of the resting spores is found, the present key characters must be retained.

In the present form the resting spores are aggregated in groups of

eight, an arrangement not previously described in the Plasmodiophoraceae. Furthermore, this is the only member of the Plasmodiophoraceae (unless *Woronina polycystis* is found to belong here) parasitic on any of the fungi. It would, therefore, seem that on the bases of resting spore characters and host the present form must be placed in a new genus. In *Tetramyxa* Goebel (1884) the resting spores are in groups of four. It seems logical, therefore, to give the present species the generic name of *Octomyxa*, and since it is limited in host range to *Achlya glomerata*, to give it the specific name, *Achlyae*.

OCTOMYXA nov. gen.

Parasite on *Achlya* causing hypertrophy of host. The infecting zoospore giving rise to a naked protoplast, which at maturity forms a large spherical sorus of zoosporangia or resting spores. Vegetative nuclear divisions of the "cruciform type." Zoosporangia thin-walled, spherical, ovoid, or slightly flattened by pressure, zoospores with one long and one short flagellum. Resting spores spherical and with a slightly thickened wall; aggregated in groups of eight, each group not enclosed in a common membrane.

Octomyxa Achlyae nov. sp.

Obligate parasite on *Achlya glomerata* Coker, causing spherical galls mostly on ends of the hyphae. Galls 50–150μ thick. Plasmodium at maturity partially or completely filling the gall and segmenting into either zoosporangia or resting spores. Zoosporangia globose to ovoid, thin-walled, variable in size, 6μ to 16μ thick. Spores discharged through small papillae which are formed only on some of the sporangia next to the host wall, the other peripheral sporangia as well as those deeper within the sorus discharging their spores through the peripheral ones; zoospores biciliate with one long posterior and one short anterior flagellum, 6–14 in a zoosporangium. Resting spores smooth walled, 2.4μ to 3.2μ, aggregated in groups of eight.

Sporangial and resting spore membranes without cellulose as shown by a negative reaction with chlor-iodide of zinc.

Collected only once and then near Hollow Rock, Durham Co., N. C., January 1936. Frances Foust, coll.

RELATIONSHIP OF OCTOMYXA

The present genus is close to *Woronina polycystis* Cornu as illustrated by Cornu (1872) if one accepts Fischer's (1882) interpretation of the zoospores as biciliate, with one long and one short cilium, and his interpretation of the resting stage as consisting of a sorus of closely packed but individual cysts instead of Cornu's interpretation of a large

spore with a warted wall. The present genus differs from *Woronina polycystis* as interpreted above: (1) in the shape of the galls, which in *Octomyxa* are usually spherical, rarely elongated, while in *W. polycystis* they are spherical or elongated; (2) in the absence of cross walls in *Octomyxa* separating the sorus from the healthy part of the hypha; (3) in the color of the cystosori, which in *Octomyxa* are colorless, while in *W. polycystis* they are brownish; and (4) in the arrangement of the spores in the sorus. In *W. polycystis* the cysto-spores or cysto-sporangia are compactly arranged in a spherical or oval mass and when crushed separate into single polygonal units, while in *Octomyxa* when the spore mass is crushed the resting spores are seen to be in clusters of eight.

This work suggests the need for a careful and complete study of *Woronina polycystis* on one of its original hosts, preferably *Achlya racemosa*. It is likely that in the forms with "spore balls" as the resting spore stage one will find the "protomitotic" type of nuclear division.

CULTURE TECHNIC

This parasite was kept in culture on its host, *Achlya glomerata*, from December 1936 to January 1939. Although several attempts were made by Miss Leitner to free the cultures of bacteria, small protozoa, and unicellular algae, these efforts were never entirely successful. The cultures were carried on in Petri dishes in charcoal water on boiled halves of hemp seed. New cultures were made by cutting off infected host threads and transferring to a hemp seed in a fresh dish. Several experiments were carried out to determine if the parasite would multiply with its host on agar. Positive results were obtained only on agar No. 12 (2% agar and 0.004% peptone). When infected threads were put on this agar, the host threads grew and the sori of the parasite developed but spores failed to mature. No attempts were made to secure transfers from the agar. It was found that the parasite was much easier to culture during cool than warm weather. In spite of this fact the parasite was lost during the month of January 1939 due to some unknown reason.

HOST RANGE

Some experiments were carried out to determine the host range of the parasite. Cultures of the following water molds were put in the dish with the parasite: *Saprolegnia ferax*, *S. megasperma*, *Achlya imperfecta*, *A. flagellata*, *A. colorata*, *A. racemosa*, *A. deBaryana*, *Aphanomyces stellatus*, *Apodachlya brachynema*, *A. minima*, and *Allomyces arbuscula*.

No infection occurred in any of the above species. From the experiments carried out it seemed that the parasite was limited to one host, *Achlya glomerata*.

DEVELOPMENT OF PARASITE

Due to the death of the parasite we have been unable to work out a complete account of the developmental stages. The account given below was worked up partly from living material and partly from whole mounts killed with Claussen's fluid and stained by Gram's gentian violet technic.

Zoospores have been observed attached to the host hyphae near the tip and also some distance back from the tip but the actual entry of the parasite into the host has not been seen. If killed in Claussen's fluid and stained with Gram's stain the nucleus of the parasite is easily distinguished from the host nuclei because of the larger size of the former. One instance was observed, however, where the spore had apparently just entered through the host wall, leaving a minute trace of cytoplasm still attached to the entrance pore but with no cyst on the outside (Pl. 47, fig. 2). A number of early infection stages, though none so convincing as the above, have been seen also without cysts. It seems, therefore, that the entire spore enters, leaving no cyst. In none of the other genera of the Plasmodiophoraceae have cysts been observed.

Though infection may occur anywhere along a hypha the galls are always formed at or near a hyphal tip. The sorus of sporangia or resting spores, however, may extend for some distance back into the unswollen part of the hypha.

As a rule a sorus develops from a single spore infection but sometimes it appears that several plasmodia may unite to form one sorus. The finding of as many as six or eight small plasmodia closely associated in one swelling of the host suggested that fusion of the plasmodia would occur. Yet a number of examples have been seen where two zoosporangial sori or a zoosporangial and a resting spore sorus both developed in the same gall. In such a case one of the zoosporangial sori was ahead of the other in development, a condition which might account for their failure to fuse. Indeed, several instances have been observed in which a smaller plasmodium was developing within a vacuole completely surrounded by a larger plasmodium.

Though instances have been observed which might be interpreted as plasmodial fusions, there seems to be more proof at present that the plasmodia do not fuse.

As the plasmodium develops the hyphal tip swells, the enlargement of the gall proceeding, as a rule, much faster than the growth of the parasite so that the former attains its mature size before the latter (Pl. 47, fig. 1a–c). The plasmodium lies in a vacuolated area connected with the host cytoplasm by numerous nearly hyaline cytoplasmic strands along which one can observe the movement of small particles toward the parasite (Pl. 47, fig. 5). As noted by Kunkel (1915) for *Spongospora subterranea*, the parasite "actually lives within the protoplasm of its host." In these earlier stages of development it is impossible to be sure just where the parasite protoplasm stops and that of the host begins.

Vegetative nuclear divisions, i.e. the divisions occurring while the plasmodium is growing, are of the protomitotic type (Pl. 47, figs. 3–4), as has been described by Nawaschin (1899), Cook (1928), Horne (1930), Webb (1935), and others for related genera.

The zoosporangial and resting spore plasmodia are indistinguishable until sometime after cleavage has begun. The cleavage process and the nuclear details in zoosporangial, zoospore and resting spore formation will be described in a later paper by Miss Whiffen.

When the zoosporangia are first recognizable they are globose or ovoid in shape and with large lumps of undigested material in the cytoplasm. Sometimes segmentation may be incomplete so that several zoosporangia are connected by narrow isthmuses. Several hours later the lumps have disappeared and the protoplasm has become evenly granular with a large central vacuole in each zoosporangium. The vacuole now pushes outward, cleaving the protoplasm into uninucleate spore origins. Meanwhile several papillae of emergence have been formed, only one papilla ever being formed on a sporangium. These are not formed on all the sporangia but on part of those in contact with the host wall and on some toward the center of the sorus. Not all of the sporangia within a given plasmodium necessarily mature simultaneously.

The sporangia with papillae in contact with the host wall open to the outside to discharge their spores while those with papillae opening into the gall discharge the spores in the gall. Emerging spores were studied by the senior author under a Zeiss water immersion lens. The spores emerge singly and slowly and after a few seconds of sluggish motion at the sporangial tip may swim away. The spores are equipped with two cilia, a long and a short one. Both cilia are attached to the spore at or near the anterior end but the short one is in front while the longer one extends backwards (Pl. 47, fig. 8). Spores with four cilia, two long and

two short, were sometimes seen, such double spores perhaps being due to incomplete segmentation. No fusion of spores was observed, though it is possible that fusions might occur.

FORMATION OF THE RESTING SPORES

The resting spores appear only in cultures several days old, following the formation of zoosporangia. As mentioned above, the zoosporangia and resting spore plasmodia are indistinguishable, so far as we know at present, until sometime after cleavage has begun. In the formation of the resting spores the spherical plasmodial mass cleaves into a number of smaller masses of protoplasm and these blocks are then segmented, as a rule, into eight uninucleate masses, each mass encysting to form a group of two tetrads of resting spores. This remarkable grouping of the resting spores suggested to the senior author the possibility of some peculiar arrangement of the spindles in the nuclear divisions preceding the formation of the resting spores (see Miss Whiffen's abstract elsewhere in this Journal). This double tetrad of spores does not always hold, for frequently one finds four normal sized spores and two larger ones making a group of six (Pl. 47, fig. 10), and again one may find nine spores, the extra ones perhaps coming from an adjacent group which would be left with seven.

After a rest period of about two weeks the resting spores are capable of germinating. Zoospores were seen emerging from the cysts, one from each cyst, but the number of cilia and the method of swimming were not determined. After several months (about 5) the resting spores lose their vitality.

DISCUSSION

The occurrence of zoosporangia as well as resting spores in the life cycle of a member of the Plasmodiophoraceae was reported for the first time by Cook (1925). Cook found that in the genus *Ligniera,* under certain conditions of moisture and temperature, the plasmodium cleaves into large segments around which a thin wall is formed, enclosing a single nucleus. Each segment comprises a zoosporangium which becomes multinucleate by successive divisions of the nucleus. Finally zoospores are cut out by cleavage of the protoplasm around each nucleus. In 1930 Cook and Schwartz made a study of *Plasmodiophora brassicae.* After germination of the resting spore, the swarm cell enters a root hair of the host and there develops into a multinucleate plasmodium. At maturity the plasmodium is cut up into a number of zoosporangia. The zoospores after their escape fuse in pairs, according

to Cook, and the diploid plasmodium thus formed gives rise to resting spores. Ledingham (1935) has reported the occurrence of the zoosporangia of *Spongospora subterranea* in the root hairs of tomato and potato seedlings. Also Ledingham (1939) has described a new genus and species, *Polymyxa graminis*, in which zoosporangia are produced. *Octomyxa Achlyae* can now be added to this list of genera in which zoosporangia as well as resting spores have been discovered in the life cycle.

Observations on the ciliation of the zoospores in the Plasmodiophoraceae are difficult, due to the rarity with which active zoospores are obtained. Woronin (1878) and Cook and Schwartz (1930) report uniciliate zoospores in *Plasmodiophora brassicae*. Cook (1928) states that in *Ligniera junci* both the swarm cells from the resting spores and the zoospores from the zoosporangia are uniciliate. In *Sorosphaera radicale* (Cook and Schwartz, 1929) and in *Sorodiscus radicicolus* (Cook, 1931) the zoospore is described as possessing a single apical flagellum about the same length as the body of the zoospore. Cook (1933) has germinated the resting spores of *Spongospora subterranea* on potato agar and observed a uniciliate zoospore. Ledingham (1934), however, has germinated the resting spores of *Plasmodiophora brassicae* and *Spongospora subterranea* and has published photomicrographs of the zoospores thus obtained and stained by Cotner's method. These zoospores are biciliate with one long and one short cilium. Ledingham (1939) also has described biciliate zoospores in *Polymyxa graminis*. The zoospores of *Octomyxa Achlyae* when studied both in the living state and in stained preparations show clearly the presence of a short and a long cilium. It thus appears that within the Plasmodiophoraceae both uniciliate and biciliate zoospores are found. A contradictory situation exists in *Plasmodiophora brassicae* and *Spongospora subterranea* where Cook reports uniciliate zoospores and Ledingham biciliate zoospores. It is possible that these two workers are dealing with different organisms.

With the exception of *Sorodiscus Karlingii* on *Chara contraria* and the possible occurrence of *Ligniera junci* on *Isoetes lacustris,* all the known species of Plasmodiophoraceae parasitize only the Spermatophytes. *Octomyxa achlyae,* therefore, is the first member of the Plasmodiophoraceae to be reported as occurring on a fungus.

SUMMARY

A new genus of the Plasmodiophoraceae, *Octomyxa,* is described based on the species *O. Achlyae*. The species is an obligate parasite on the hyphae of *Achlya glomerata,* causing the formation of gall-like swellings

on the hyphal ends. Infection is by single spores which enter the hyphae without leaving a cyst. The spore is carried to the hyphal tip where it develops into a multinucleate plasmodium. Vegetative nuclear divisions are of the protomitotic type characteristic of the Plasmodiophoraceae. The early formed plasmodia develop into sori of zoosporangia which in turn give rise to zoospores. The zoospores are biciliate with one long and one short cilium. The later formed plasmodia form sori of resting spores. These are found as a rule in groups of eight, each group being made up of two tetrads of resting spores.

LITERATURE CITED

COOK, W. R. IVIMEY. 1926 The Genus *Ligniera* Maire and Tison. Trans. Brit. Myc. Soc. **11**: 196–213.

1928 The Methods of Nuclear Division in the Plasmodiophorales. Ann. Bot. **42**: 347–377.

1931 The Life History of *Sorodiscus radicicolus* sp. nov. Ann. Mycol. **29**: 313–324.

1933 A Monograph of the Plasmodiophorales. Archiv für Protistenkunde **80**: 179–254.

COOK, W. R. IVIMEY, AND E. J. SCHWARTZ. 1929 The Life History of *Sorosphaera radicale* sp. nov. Ann. Bot. **43**: 81–88.

1930 The Life History, Cytology and Method of Infection of *Plasmodiophora brassicae* Wor. Phil. Trans. Roy. Soc., Ser. B, **218**: 283–314.

CORNU, M. 1872 Monographie des Saprolégniées. Ann. Sci. Nat. Bot. Ser. 5, **15**: 1–198.

COUCH, J. N. 1932 The Development of the Sexual Organs in *Leptolegnia caudata*. Amer. Jour. Bot. **19**: 584–599.

FISCHER, A. 1882 Untersuchungen über die Parasiten der Saprolegnieen. Jahrb. für Wiss. Bot. **13**: 286–371.

GOEBEL, K. 1884 *Tetramyxa parasitica*. Flora **67**: 517–521.

HORNE, A. S. 1930 Nuclear Division in the Plasmodiophorales. Ann. Bot. **44**: 199–231.

KUNKEL, L. O. 1915 A Contribution to the Life History of *Spongospora subterranea*. Jour. Agric. Res. **4**: 265–278.

LEDINGHAM, G. A. 1934 Zoospore Ciliation in the Plasmodiophorales. Nature **133**: 534.

1935 Occurrence of Zoosporangia in *Spongospora subterranea* (Wallroth) Lagerheim. Nature **135**: 394.

1939 Studies on *Polymyxa graminis* n. gen. n. sp.,a Plasmodiophoraceous Root Parasite of Wheat. Canadian Jour. Res. **17**: 38–51.

Nawaschin, S. 1899 Beobachtungen über den feineren Bau und Umwandlungen von *Plasmodiophora Brassicae* Woron., im Laufe ihres intracellularen Lebens. Flora **86**: 404–427.

PALM, B. T., AND MYRLE BURK. 1933 The Taxonomy of the Plasmodiophoraceae. Archiv für Protistenkunde **79**: 263–276.

WEBB, PHILIP C. R. 1935 The Cytology and Life-history of *Sorosphaera Veronicae*. Ann. Bot. **49**: 41–52.

WORONIN, M. 1878 *Plasmodiophora Brassicae*, Urheber der Kohlpflanzen-Hernie. Jahrb. für Wiss. Bot. **11**: 548–574.

PLATE 47

Octomyxa Achlyae

(All figures were drawn by J. N. Couch and inked by Else R. Couch except figs. 2-4 which were inked by Alma Whiffen)

Fig. 1. Habit sketch of parasite on threads of *Achlya glomerata* showing successive stages in development of sporangial sorus, a–e and two sori of resting bodies, f, g. ×142.

Fig. 2. Early infection stage showing the parasite nucleus just after entering the host. Drawn from stained material prepared by Jean Leitner. ×1620.

Figs. 3–4. Binucleate plasmodia with parasite nuclei undergoing protomitotic division. Drawn from stained material prepared by Jean Leitner. ×1620.

Fig. 5. Enlarged view of living parasite plasmodium in tip of hypha. ×1000.

Fig. 6. Nearly mature sporangia. ×1310.

Fig. 7. Sporangia with emerging spores. ×635.

Fig. 8. Zoospores. Note long and short cilium on spores; also note double spore with two long and two short cilia. ×635.

Fig. 9. Sorus of resting spores. ×585.

Fig. 10. Resting spores from spore ball as in Fig. 9. Note spores usually in groups of eight or four. ×1000.

PLATE 48

Octomyxa Achlyae

Photographs by J. N. Couch

Fig. 1. Living spherical plasmodium which has attained mature size in swollen tip of host hypha. Picture is under developed in printing to prevent opaque plasmodium from coming out jet black. Faint cytoplasmic strands of host can be seen on left attached to plasmodium. At this stage the plasmodium contains many small vacuoles, which show up faintly on right as lighter spots. We do not know if such a plasmodium forms zoosporangia or resting spores. Note absence of cross wall at base of gall. ×516.

Fig. 2. Exceptional sorus of zoosporangia which does not fill gall. Note finely granular cytoplasm of zoosporangia and absence of cross wall separating gall from hypha. Cleavage of protoplasm to form zoospores has already begun. ×720.

Fig. 3. Zoospore with one short and one long flagellum. Killed on slide in water by exposure for 30 seconds to 1% osmic acid fumes and stained by addition of a few tiny crystals of gentian violet. ×2000.

Fig. 4. Showing three sori. Above, zoosporangial sorus, partly crushed by cover slip. Note zoosporangia are somewhat polygonal from pressure. Middle to right, immature resting spore sorus. Note absence of cross wall in hypha (the apparent basal membrane is edge of host wall). Lower left, resting spore sorus, slightly crushed by cover slip. Mounted in glycerine with a trace of lactophenol and coton blue. ×1170.

Fig. 5. Greater enlargement of lower resting spore sorus in Fig. 4. Note grouping of resting spores. In some of the clusters eight spores can be counted. ×1520.

PLATE 47

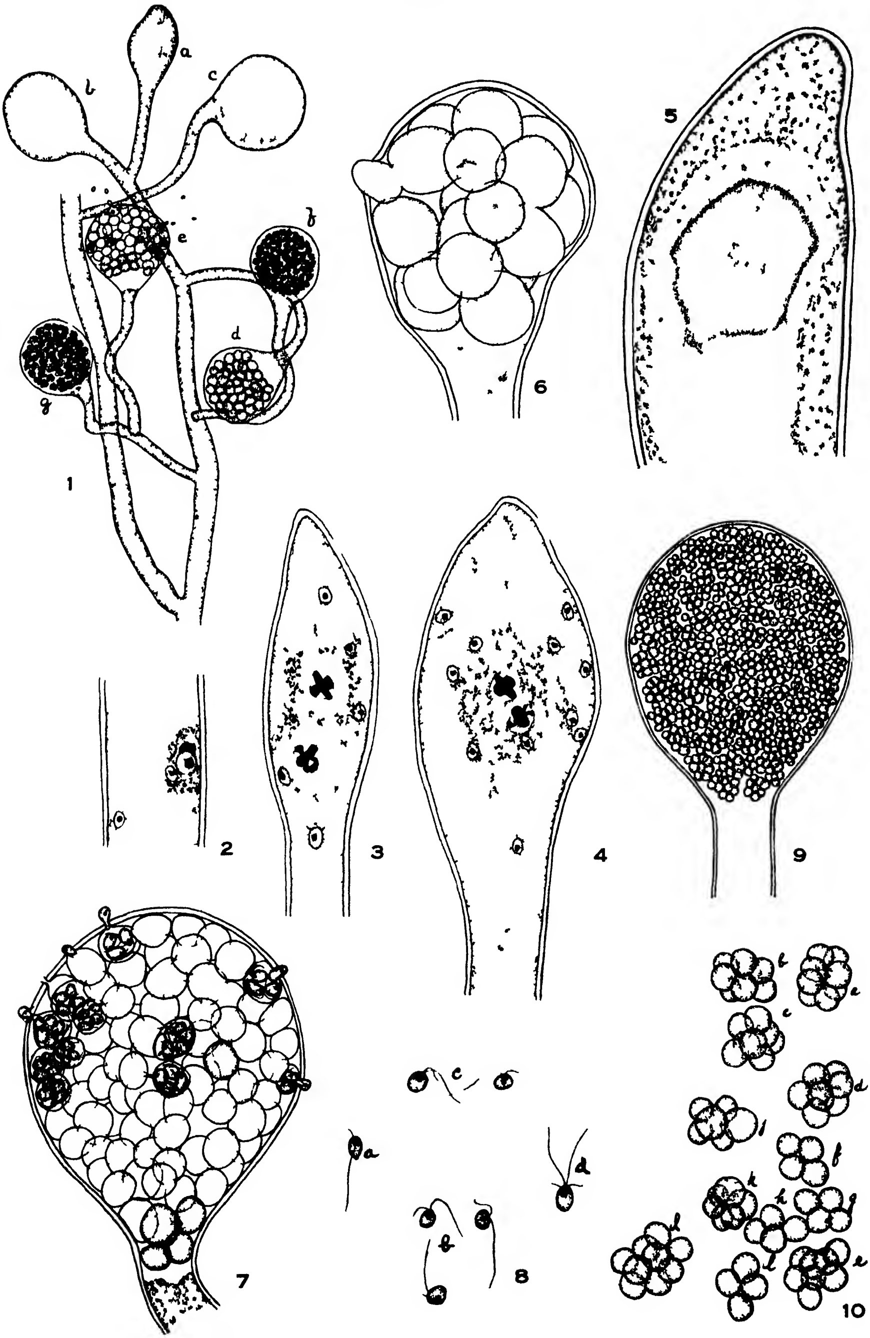


PLATE 48

HETEROTHALLISM IN THE CHYTRIDIALES

By John N. Couch

Plate 49

Heterothallism or the separation of the sex factors in two different thalli has now been reported in all the major groups of fungi except the Chytridiales. In our laboratory we have developed technics for culturing some of the chytrids and are now able for the first time to report this condition in two different genera of this order.

The species to be considered first is an obligate parasite on *Achlya flagellata* and has been incorrectly placed in the genus *Woronina* by Cornu (1872), Fischer (1882), and the writer (1939). It was first illustrated by Pringsheim (1860) but the accurately drawn, uniflagellate zoospores were misinterpreted by him as the male cells of a sterile *Achlya*. Cornu did not illustrate the zoospores of *Woronina polycystis* but Fischer and more recently Cook showed them with two flagella. It has therefore been assumed that Pringsheim was in error when he showed a single flagellum on the spore. However, I have found the same genus, perhaps the same species of fungus as that illustrated by Pringsheim, and now have three strains of it in culture and can positively verify the correctness of Pringsheim's figures of the uniflagellate zoospores. Since this fungus is very distinct from *Woronina* as illustrated by Cornu, Fischer, and Cook, and does not fit in with any known genus, it is necessary to give it a new generic name. It seems very appropriate to name it in honor of Pringsheim.

PRINGSHEIMIELLA gen. nov.

Obligate endophytic parasites on Saprolegniaceae. Thalli from zoospores multiplying in host hyphae. Zoosporangial sori formed in the ends of host hyphae and resembling in superficial appearance and development the host zoosporangia. Sporangia globose or polygonal from pressure, each sporangium with an emergence papilla. Zoospores posteriorly uniflagellate, very minute. Resting bodies unicellular, spherical, brownish when mature, one to several formed within a larger polygonal or irregularly shaped cell. Zoosporangial and resting spore membranes bluish purple with chlor-iodide of zinc.

Pringsheimiella dioica sp. nov.

Infection by zoospores which leave cyst on host wall. Thalli from zoospores multiplying in host hyphae and carried by plasma currents of host to the distal parts of hyphae. Sporangia formed in cylindrical compartments, the latter resembling in shape, size, and position the sporangia of the host. Compartments separated from healthy host hypha by cross wall. Each sporangium in a sorus developing from a separate thallus and forming an emergence papilla upon maturity. Sporangia spherical to oval or polygonal from pressure, 16–21μ thick, with a cellulose membrane. Zoospores very minute, 1.8–3 x 3μ, spherical, or slightly elongated, with one minute glistening globule, and a single posterior flagellum, moving actively within the sporangium before discharge and swimming away after a brief pause at the sporangial mouth. Resting bodies (zygotes) formed only where two sexually opposite or sexually compatible strains are brought together, spherical, 15–17μ thick, golden brown with an eccentric globule and a minutely rough or reticulate membrane, formed within a larger cell which is polygonal from pressure. Containing cell 25–30μ thick; sometimes larger and containing as many as six zygotes. Germinating by uniflagellate zoospores after six weeks' rest.

Collected several times from North and South Carolina. Near Florence, S. C., June 1929, J. N. and A. B. Couch, colls. On *Achlya* sp. near Chapel Hill, N. C., 1934, J. N. Couch, coll.; Patterson Pasture, Chapel Hill, N. C., strain no. 169 on *Achlya flagellata*, March 1938, J. N. Couch, coll.; University Lake, Chapel Hill, N. C., on *Achlya flagellata*, strain no. 218, Jan. 1939, Ward and Shanor, colls.

Pringsheimiella has been known in the past only in its asexual stage as illustrated by Pringsheim (1860). In this stage the parasite produces vast numbers of spherical or polygonal sporangia 16–21μ thick, which develop in special compartments in the distal parts of the host hyphae. A mature sorus of sporangia has an appearance suggestive of a sporangium of *Dictyuchus* (figs. 1–3). Upon maturity, however, each sporangium gives rise to numerous very minute zoospores 1.8–3 x 3μ, slightly elongate or spherical, with a single posterior flagellum and the characteristic glistening globule. The spores emerge, swim in the typical chytrid fashion and after a swimming period reinfect the host. Strain number 169 has been in the laboratory over eighteen months and in all cultures has produced only zoosporangia when grown alone.

Last January one of our students turned over to me what appeared to be a form of *Dictyuchus* with particularly large zoosporangia (fig. 1). Careful study, however, showed that this was another strain of *Achlya flagellata* infected with *Pringsheimiella*. This strain proved to be the

complementary sexual strain to no. 169 and was designated 218. It has been in culture since last January (now October 26) and has produced only zoosporangia when grown alone on its host.

In trying to separate the host from its parasite by culturing the *Achlya* on agar #13 it was observed that parasite strains 169 and 218 showed entirely different growth characters on agar. Number 169 formed only a few parasite zoosporangia on agar but the plasmodial stage continued to multiply as the hyphae grew, being easily detected near the hyphal tips as darker granular cytoplasm. In this strain one could isolate the parasite on its host even after the culture had covered the dish by cutting out hyphae containing the dark areas of protoplasm. Strain 218 produced many parasite zoosporangia during the first three days on agar #13 but after the third day it was impossible to recover the parasite from the hyphae, the parasite having apparently used itself up in sporangial production.

These physiological differences were evident when the two parasites were cultured on their *Achlya* hosts on sterile hemp seed in water. Under such conditions strain 169 produced fewer sporangia than strain 218 and they were as a rule thinner and longer than in strain 218. The two hosts though belonging to the species *Achlya flagellata* also showed slight physiological differences. *Achlya* strain 169 grew more slowly on agar no. 13 than strain 218 and appeared to be a less vigorous grower on hemp seed in water. When parasite strain 169 was transferred by spores to pure cultures of *Achlya* 218 and parasite strain 218 to *Achlya* strain 169, and then cultured on agar each parasite retained its physiological characters as though growing on its original host.

Such physiological differences in the parasites suggested the possibility of different sexual strains. To test the possibility of such a condition a number of hemp seed cultures of *Achlya* 169 infected with parasite 169 and of *Achlya* 218 infected with parasite 218 were made. About thirty-six hours later when both parasites were producing abundant zoospores a culture of 169 and one of 218 were transferred to a fresh dish and so placed that the *Achlya* threads of the two intermingled along one side of each culture. Three other such "crosses" were made in separate dishes at the same time.

On examination two days after crossing, each of the crosses showed, particularly in the regions where the threads of the two strains intermingled, abundant resting bodies (figs. 4, 5). These were formed either in the zoosporangial sori intermingled with zoosporangia or in sori containing only resting bodies. After three days the resting bodies

were mature (fig. 6). They are spherical, 13–17μ thick, with an eccentric fat globule, and a double-layered wall, the outer layer being minutely spiny or reticulate. Each zygote is contained in a large thick-walled compartment which is about twice the size of the sporangia, 24–32μ, and usually polygonal with rounded edges, rarely nearly spherical. As a rule one zygote is formed in a compartment but sometimes as many as six may be formed in one compartment.

After a rest of six weeks the zygotes germinate by the formation of uniciliate zoospores.

The details of development, etc., will be published in a later paper.

The next species to be considered belongs in the *Rhizidiaceae* and is now known as *Rhizophlyctis rosea* (de Bary & Wor.) Fischer. (For a detailed description and illustrations see Miss Ward's paper elsewhere in this journal.) This species was first collected in this region by the writer in November 1938 and has since been found from many localities by several workers in our laboratory. In March 1939 the writer found an old "Land Posted" cardboard sign in a drainage ditch near The Caves on which the fungus was growing abundantly and producing many resting spores. Since these resting spores had not appeared in any of our other collections it was suspected that a heterothallic condition existed and that both sexual strains were present on the cardboard.

To test this possibility twenty single sporangia were dissected out (March 10 and 11) under a dissecting binocular microscope (×40) and put in separate dishes in charcoal water on boiled grass leaves, boiled corn leaves, boiled filter paper, and boiled cardboard. On examination March 24, 1939, nos. 1, 2, 6, 7 showed growth of new sporangia on grass and corn but no growth on paper. No growth was seen in any of the 16 others, in fact most of the sporangia had not discharged their spores. On examination several days later no. 7 had died but no. 20 had discharged zoospores and many new sporangia were formed. In none of the four cultures, nos. 1, 2, 6, 20, were any resting bodies formed even after several weeks and several transfers.

All possible crosses were then made between these four strains as follows: 1x2, 1x6, 1x20, 2x6, 2x20, 6x20, but only when strain 2 was crossed with one of the others were resting bodies formed. Similar crosses have been made several times with the same results. It seems logical, therefore, to conclude that strain 2 belongs to one sex and the other three strains, nos. 1, 6, 20, belong to another sex. Unfortunately, strain no. 2 was lost during the summer months before detailed studies

could be made to determine how, or even if, fusion occurred in the formation of the resting spores.

SUMMARY

It has been found that the uniciliate Chytrid parasite first illustrated and partly described by Pringsheim as the antheridial stage of *Achlya dioica* and later erroneously considered as *Woronina* by Cornu, Fischer, and others is heterothallic. If two of the opposing strains are cultured on their hosts in the same Petri dish with or without the host hyphae in contact, spherical, thick-walled resting bodies are formed. These resting bodies resemble those of *Rozella* and *Pleolpidium*. Since this parasite is very distinct from *Woronina polycystis* as interpreted by Cornu and Fischer and unlike any known genus it is being described under a new name *Pringsheimiella* in honor of Pringsheim who first accurately described but misinterpreted its nature.

Rhizophlyctis rosea (de B. & Wor.) Fischer is also heterothallic. Four single sporangial strains were isolated, none of which produced resting bodies when grown alone. Crosses made in all possible ways indicated that one of the single sporangial cultures was of one sex while the three remaining were of the opposite sex.

LITERATURE CITED

CORNU, M. 1872 Monographie des Saprolégniées. Ann. Sci. Nat. Bot. Ser. 3, 15: 1–198.

COUCH, J. N. 1939 Technic for collection, isolation and culture of Chytrids. Journ. Eli. Mit. Sci. Soc. 55: 208–214.

FISCHER, A. 1882 Untersuchungen über die Parasiten der Saprolegnieen. Jahrb. für Wiss. Bot. 13: 286–371.

PRINGSHEIM, N. 1860 Beiträge zur Morphologie und Systematic der Algen. IV. Nachträge zur Morphologie der Saprolegnieen. Jahrb. f. Wiss. Bot. 2: 205–236.

EXPLANATION OF PLATE 49

Pringsheimiella dioica

Photographs by author

Fig. 1. Sori of zoosporangia of strain 218. Note striking similarity of sori to sporangia of *Dictyuchus*. The three sori one to left and two in basipetal arrangement on same thread in center show sporangia nearly mature. In sorus just below center sporangia are in "sporangial origin" stage, corresponding to spore origin stage in sporangium of host. On right of center two long sori with empty sporangia and sporangia with spores. No sporangia of host are visible. ×100.

Fig. 2. Sori of strain 169. Note sporangia in row at center and very large sori, upper right. Some sporangia empty others with spores. ×150.

Fig. 3. Distal part of sorus strain 169. Some empty sporangia and others nearly mature. Note two papillae on left about center ×1050.

Fig. 4. Strains 169 × 218 showing empty zoosporangia of parasites; small round zygotes within their irregular containing cells; gemmae of host and at bottom right two oogonia of host are faintly visible. Note zygotes of parasite are formed both in cylindrical and spherical sori, usually along with zoosporangia. ×150

Fig. 5. Stages in development of three zygotes, two near center and one on right. Note young zygotes are partly surrounded by large vacuole. The smaller spherical bodies are nearly mature zoosporangia ×700.

Fig. 6. Two zygotes, with centric or slightly eccentric globule, thick wall and large containing cell. Also note empty zoosporangia in same sorus. ×1050.

PLATE 49